Autolab TWINGLE/ SPRINGLE Data Acquisition 4.4

User manual SPR

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Welcome

The Autolab Twingle and Autolab Springle are Surface Plasmon Resonance instruments used for analysis of biomolecular interactions in real time without labelling. Our systems also provide the possibility of simultaneous electrochemical measurement with a separate potentiostat / galvanostat. Surface Plasmon Resonance (SPR) has become a standard tool in life sciences and pharmaceutical research laboratories. The study and characterisation of molecular interactions is essential to explore the structure-function relationships of biomolecules and to aid our understanding of biological systems in life sciences, like antibody-antigen, ligand-receptor, protein-nucleic acid, cell adhesion and drug development. With the option of electrochemistry, an extra tool is available for the study of enzymes, ion channels, membrane proteins, polymer layers, polymerisation and polymer-biomolecular interactions.
SPR provides quantitative information, which can be used to determine reaction kinetics and affinity constants for molecular interactions, as well as the active concentration of biomolecules in solution. It also provides qualitative information and allows small-molecule screening to epitope mapping and complex assembly studies.

The SPR technique is related to the nature of the surface plasmon. Methods of optical excitation and basic properties of surface plasmon resonance that are important in the sensor application are summarized in this manual.

The optical detection principle of the Autolab SPR instrument has been derived from technology developed at the University of Twente, The Netherlands, which was supported financially by the Dutch Foundation of Technical Sciences (STW). The hardware and software of the Autolab instrument was developed by Metrohm Autolab BV.
Another SPR system is the Autolab ESPRIT, a fully automated double channel SPR system.

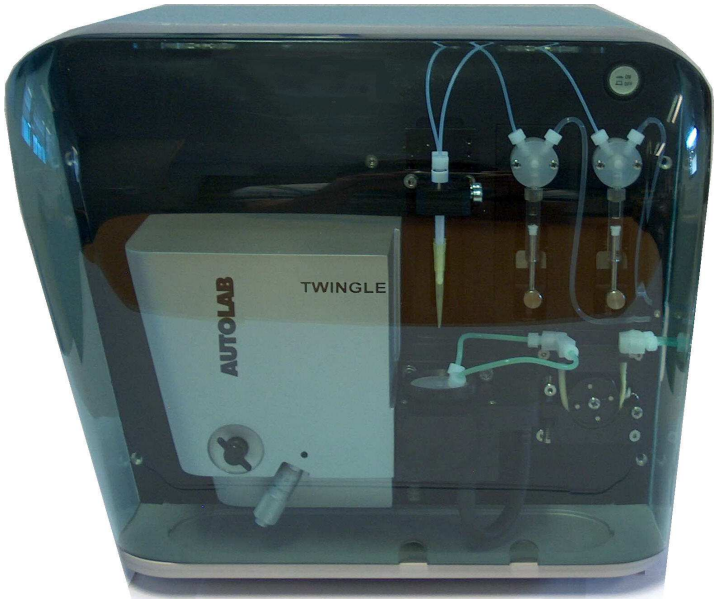
The user manual consists of eleven chapters:

- Chapter 1 describes the hardware installation
- Chapter 2 describes the software installation
- Chapter 3 describes a ‘getting started’ experiment for the TWINGLE
- Chapter 4 describes a ‘getting started’ experiment for the SPRINGLE
- Chapter 5 describes the data acquisition software in detail
- Chapter 6 provides detailed information regarding the sequence editor
- Chapter 7 describes detailed information regarding the automation
- Chapter 8 explains the theoretical background on the Surface Plasmon Resonance technique
- Chapter 9 discusses the maintenance of the SPR instrument
- Chapter 10 shows some trouble shooting

- Chapter 11 has a list of all figures of this document
- Chapter 12 is the index of this document

Important notice:

The Autolab SPR instruments are developed as a research instrument by Metrohm Autolab B.V. However, Metrohm Autolab B.V. can never be held responsible for the outcome of results, or the interpretation of results, measured with Autolab instruments.



Safety rules

For personal safety and to prevent any unnecessary damage to the Autolab TWINGLE/SPRINGLE, please read and take note of the following safety rules and precautions.

Failure to follow these instructions when using the instrument may cause unsafe operation or severe injury. Metrohm Autolab B.V. is not liable for any damage caused by not complying with the following rules and precautions.

** Instrument precautions;*

- This instrument was designed for use in laboratories and should not be used in rooms with high air humidity or where there is lot of dust. It is not meant to be stored or to be used outdoors. High levels of moisture and high concentrations of dust will cause leakage currents in the instrument. This can result in a risk of electrical shock and may cause fire.

- The manufacturers warranty is only valid for use in the permitted environments, as stated.

- Like most electronic equipment, the Autolab TWINGLE/SPRINGLE requires air to cool the electronics. If the air supply is restricted, this may result in a fire. Do not cover or block the air vents of the instrument.

- If the instrument is brought from a cold environment into a warm room, do not switch on the instrument until it has warmed up. Condensed water needs to be able to evaporate.

- Do not expose the instrument to damp or wet conditions.

- Do not place the instrument in direct sunlight or anywhere where it is likely to be exposed to additional external heat sources, except a thermostatted water bath.

- Because the instrument works with a scanning mirror and other fixed mirrors, place the instrument on a stable levelled table or lab bench. Do not lean on the instrument or table during measurements. Do not

put the instrument in a position where it is subjected to vibrations, it will harm the mirror calibration and, thus, the measurements.

- It is recommended to fill all tubes with milli-Q or demineralized water to prevent bacterial growth and salt precipitation in the tubes when the autosampler is shut down for a period shorter than one week. For longer periods, it is recommended to fill the tubes with 0.05 % sodium azide in milli-Q or to remove the solution from all tubing to prevent bacterial growth.

** Personal precautions;*

- The Autolab TWINGLE/SPRINGLE instrument has a lift. Samples can be aspirated and dispensed by the pipette tip. Nothing should impede the movement of the pipette tip, injury may result.

- Never look directly into the laser beam or reflections of the laser, failure to follow this instruction may seriously damage your eyes.

- The peristaltic pump is an open system. Do not put anything near the pump. Keep the pump free from interference.

- Do not allow untrained personnel to operate the instrument without supervision.

** Electrical hazards;*

- There are no user-serviceable parts inside. Only factory qualified personnel should service the instrument.

- Removal of front or back panels may expose potentially dangerous voltages. Always disconnect the instrument from all power sources before removing protective panels.

- Replace blown fuses only with new fuses in size and rating that are stipulated near the fuse panel holder and in the manual.

- The power cord should be placed so that it cannot be damaged. The main cable should not be bent, laid over sharp edges, walked over or exposed to any chemicals. If the insulation on the main cable has been damaged, this may cause electric shocks and /or fire.

- Replace or repair faulty or frayed insulation on power cords and control cables.

- Replace control cables only with original spare parts. When replacing a power cord, use only approved type consistent the regulations.

- Check all connected equipment for proper grounding. Do not attempt to move the instrument with power cords connected.

- This instrument may only be connected to a power supply that has the voltage and frequency stated on the type plate. It may only be connected to the power supply using the power cable provided. Incorrect voltages may damage the instrument.

- Disconnect the power cord during thunderstorms. Voltage surges from lightning strikes or other causes may damage the instrument through the main power supply.

Customer service

Metrohm Autolab B.V. and its worldwide network of distributors provide you with instrument service and help with technical questions. If you need assistance, please contact your local representative.
On our web page, www.Metrohm-Autolab.com, we maintain an up to date list with address details of our distributors.

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Chapter 1

1 – Hardware Installation.

1.1 – Index.

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1.2 – Introduction.

In this chapter the installation of the hardware is described. All necessary cables and accessories are supplied with the TWINGLE instrument.

1.3 – Computer requirements.

The following minimum computer hardware specifications are required:

- An IBM compatible computer
- 1 GHz Pentium 4 processor, preferably from Intel®
- sVGA graphics card with minimal 800 x 600 pixels resolution
- 512 Mb RAM memory
- 3 Gb free HDU space
- Microsoft Windows 2000 or XP
- Microsoft Vista requires 2 Gb RAM memory (remark; folder position installation direct in C:\, not in 'program files" folder!)
- One free RS232 serial communication port
- One additional free RS232 com port is required for the optional waterbath control

One USB port has to be available if an Autolab with GPES/NOVA software has to be installed on the same computer.

1.4 – Autolab TWINGLE/SPRINGLE Hardware.

Power Supply	100-240V +/- 10% (auto select)
Power-Line frequency	47-63 Hz
Power consumption	120 VA max.
Fuses	2 * 800 mA slow blow
Operating Environment	+10 °C to +40 °C ambient temperature < 80% relative humidity
Storage environment	+10 °C to +40 °C ambient temperature
Dimensions (W x H x D)	330mm x 400mm x 360mm
Weight	24 kg
Warm-up time	60 minutes
Remote interface	RS232
Wave length light	670 nm

1.5 – Specifications.

Table 1. Specification of the Autolab TWINGLE system.

Technical Specification	*Definition*	
Measuring principle	Surface plasmon resonance	
Transducer principle	Scanning mirror	
Liquid handling	Cuvette system	
Parallel channels	Two	
Fixed wavelength	670 nm	
Sample loading and injection	Manual or semi-automatic	
Mixing	Continuous wall jet	
Pumps	2 x Syringe pump 1 x peristaltic pump	
Flow rate range	0.8 µl/s – 227.3 µl/s 30 µl/s -130 µl/s	Syringe pump Peristaltic pump
Sample volume	20 µl - 150 µl	
Offset of SPR angle by spindle	62^{o} - 78^{o}	
Dynamic range	4000 m^{o}	
Angle resolution	< 0.02 m^{o}	
Minimum molecular weight	180 D	
Association constant range	10^{3} – 10^{7} $M^{-1}s^{-1}$	
Dissociation constant range	10^{-5} – 10^{-1} s^{-1}	
Equilibrium affinity	10^{4} – 10^{10} M^{-1}	
Concentration range	10^{-11} – 10^{-3} M	
Refractive index	1.26 – 1.38 (standard), optional 1.32 - 1.44 or 1.40 - 1.52	
Refractive index resolution	< 1.10^{-7}	
Measuring frequency	76 Hz	
Time interval range	0.1 s – 300 s	
Baseline noise	0.1m^{o} during a measurement time interval of 1s	
Sensors	Gold coated glass disk	
Standard supplied cuvette	Combined Electrochemistry and SPR or SPR only (no electrochemistry)	
Extra cuvette slider	Biacore sensor-chip adaptor (optional)	

Spincoater	To spincoat standard gold disks, 100-10.000 rpm (optional)
Weight	23 kg
Dimensions (H x W x D)	330mm x 400mm x 360mm
Interface	RS 232
Power requirements	170 W, 100 - 240 V, 50/60 Hz

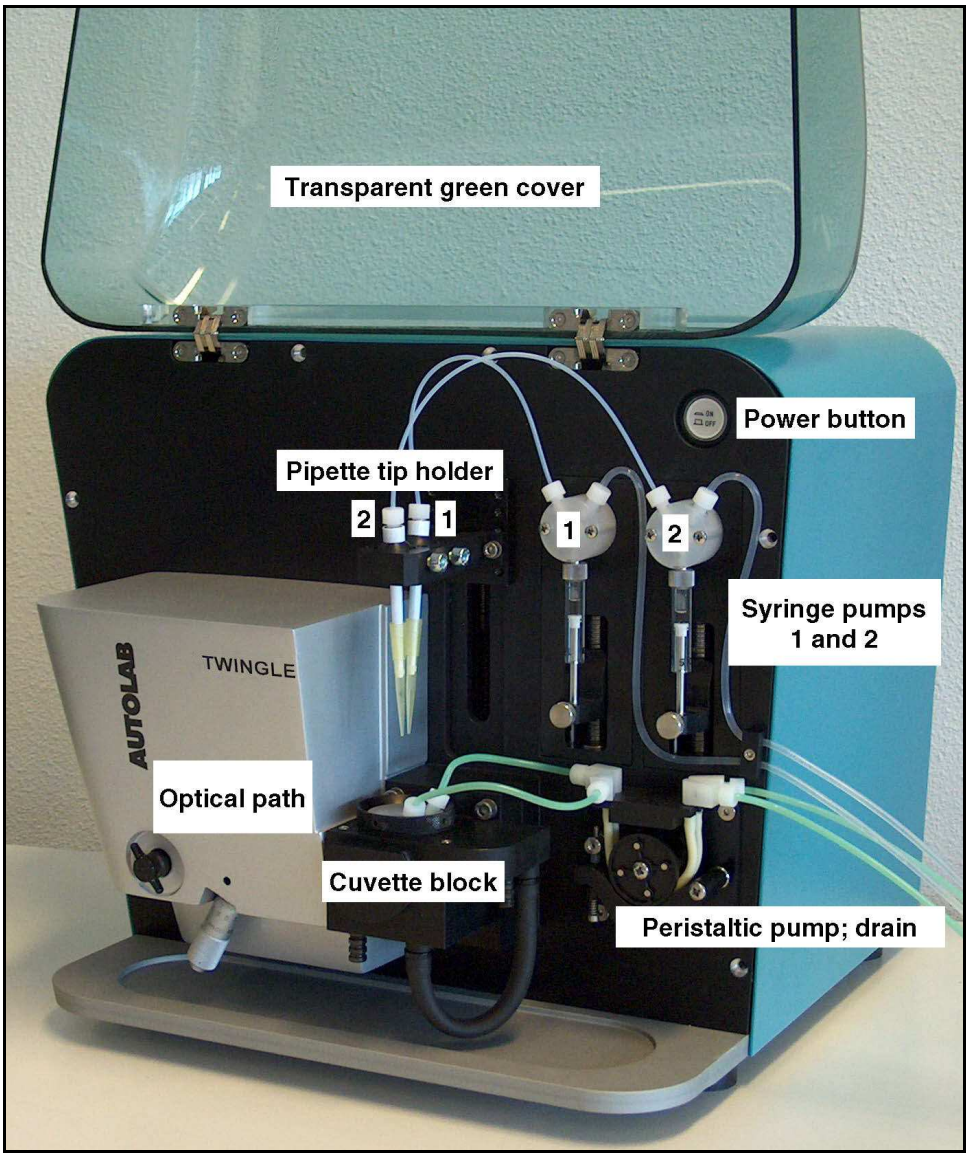

Figure 1.1 – A. The Autolab TWINGLE.

Table 2. Specification of the Autolab SPRINGLE system.

Technical Specification	*Definition*	
Measuring principle	Surface plasmon resonance	
Transducer principle	Scanning mirror	
Liquid handling	Cuvette system	
Parallel channels	One	
Fixed wavelength	670 nm	
Sample loading and injection	Manual or semi-automatic	
Mixing	Continuous wall jet	
Pumps	1 x Syringe pump 1 x peristaltic pump	
Flow rate range	0.8 µl/s – 227.3 µl/s 30 µl/s -130 µl/s	Syringe pump Peristaltic pump
Sample volume	20 µl - 150 µl	
Offset of SPR angle by spindle	62^{o} - 78^{o}	
Dynamic range	4000 m^{o}	
Angle resolution	< 0.02 m^{o}	
Minimum molecular weight	180 D	
Association constant range	10^{3} – 10^{7} $M^{-1}s^{-1}$	
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Concentration range	10^{-11} – 10^{-3} M	
Refractive index	1.26 – 1.38 (standard), optional 1.32 - 1.44 or 1.40 - 1.52	
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Measuring frequency	76 Hz	
Time interval range	0.1 s – 300 s	
Baseline noise	0.1m^{o} during a measurement time interval of 1s	
Sensors	Gold coated glass disk	
Standard supplied cuvette	Combined Electrochemistry and SPR	
Extra cuvette slider	Biacore sensor-chip adaptor (optional)	
Extra cuvette	SPR only (no electrochemistry) (optional)	

Spincoater	To spincoat standard gold disks, 100-10.000 rpm (optional)
Weight	23 kg
Dimensions (H x W x D)	330mm x 400mm x 360mm
Interface	RS 232
Power requirements	170 W, 100 - 240 V, 50/60 Hz

Figure 1.1 – B. The Autolab SPRINGLE.

1.6 – Hardware Installation.

The rear panel of the TWINGLE/SPRINGLE instruments shows a number of connectors. The layout is shown below.

Figure 1.2 – Back panel of the TWINGLE.

The main entry on the left side holds two fuses (both 800mA slow blow) and the power switch.
The functions and signals of the other connectors are described below.

<u>BNC connectors.</u>

- Trigger
  A signal output used to trigger an oscilloscope for monitoring the intensity signals.

- Intensity1
  Output of the amplified photodiode signal of channel 1, output impedance is < 1Ω, the signal varies between 0V (total absorption) and +10V (total reflection).

- Intensity2 (Twingle only)
  Output of the amplified photodiode signal of channel 2, output impedance is < 1Ω, the signal varies between 0V (total absorption) and +10V (total reflection).

- SPR1
  This signal is an analogue representation of the SPR angle measured on channel 1, output impedance is < 1Ω, and the signal varies between 0V and -10V, an SPR angle of 0 degrees equals -5V on this output.

- SPR2 (Twingle only)
  This signal is an analogue representation of the SPR angle measured on channel 2, output impedance is < 1Ω, and the signal varies between 0V and -10V, an SPR angle of 0 degrees equals -5V on this output. This BNC connector is used to connect to the

Potentiostat/Galvanostat to record SPR data into the NOVA (GPES) software.

Sub D connectors.

- Monitor
  This output is used for service purposes only. It connects to a standard VGA screen to monitor activity and error messages on the internal computer in the TWINGLE/SPRINGLE.

- Service
  For service use only.

- Therm.
  Not in use.

- COM
  RS232 communication links to the computer. Please use the supplied RS232C cable, other cables may not operate properly. Note: Some SERIAL to USB converters have not enough internal memory to handle data transport.

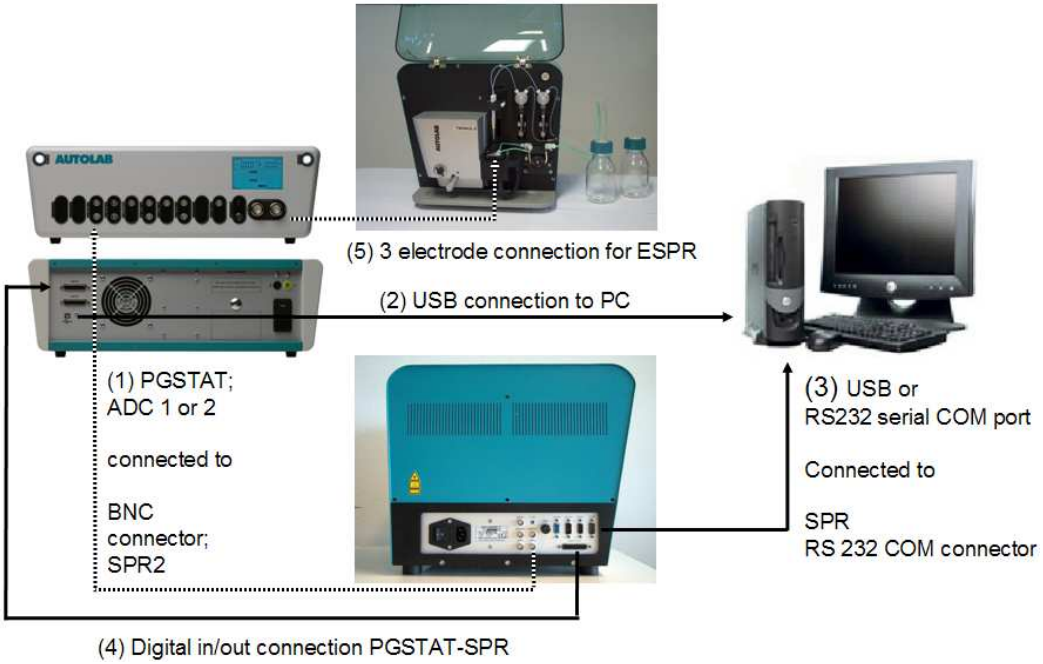
- Digital in/out
  This connector contains 24 free programmable TTL compatible digital inputs and/or outputs. It can be used to connect third party instruments to SPR, for example autosampler, FIA instruments or HPLC instruments. Automated control of Electrochemical techniques in combination with SPR is performed with a special cable connection between the two systems DIO ports, SPR system and PGSTAT.

- Mode
  For service use only.

- GND
  This banana socket is connected to the internal instrument analogue ground and indirectly connects to the protective earth. This socket is only to be used as a ground terminal for an oscilloscope for service engineers.

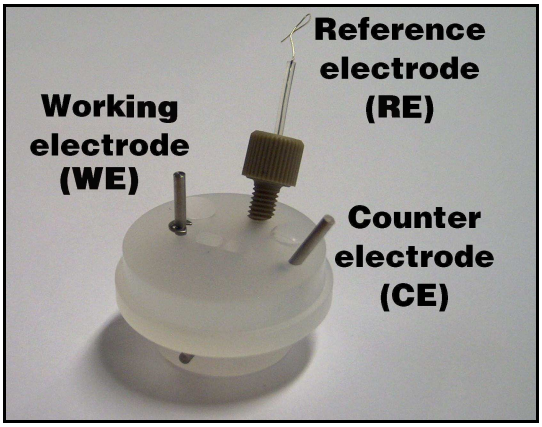
1.7 – SPR and ESPR setup.

When using the system as a stand-alone SPR system, connection with the supplied serial cable (see figure 1.3, number 3) to the PC is enough.
In order to perform combined electrochemical and SPR measurements (i.e. ESPR), the following setup of the system is required;



1. Connect the coax cable from the BNC connector, SPR 2 (Twingle) or SPR 1(Springle), of the SPR instrument to the ADC 1 or 2 of the PGSTAT,
2. Connect the Autolab USB cable to the PC,
3. Connect the serial cable from the COM-port of the SPR instrument, to a free COM port on the PC,
4. For automated control of ESPR experiments, connect the DIO ports of SPR and PGSTAT,
5. Connect cell cables to electrodes in the cuvette.

A1. ESPR cuvette



A2. SPR cuvette

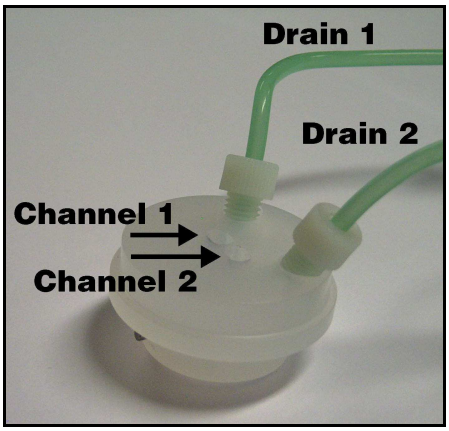
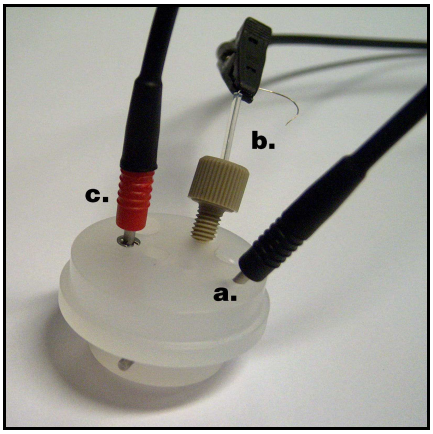


Figure 1.3 A – The Electrochemical cuvette and the normal SPR cuvette.

B.



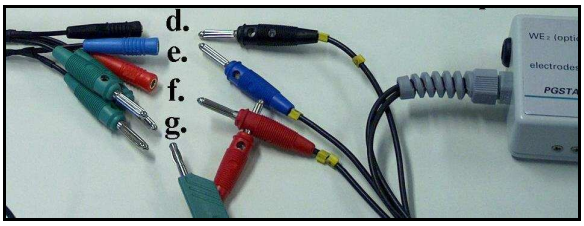


Figure 1.3 B – The Electrochemical cuvette.

A. 1. The electrochemical SPR TWINGLE cuvette with the three electrode connections, WE, RE and CE.
2. The 'normal' SPR cuvette for the Twingle.
Compared with the SPRINGLE cuvettes, the SPRINGLE will have ONE channel less and ONE drain less.

B. Connecting the cuvette electrodes to the potentiostat.
   a. Counter electrode wire for the platinum bar (CE). (black)
   b. Reference electrode wire for the Ag/AgCl RE.(blue)
   c. Working electrode wire for the gold contact (WE).(red)
   d. Connecting the CE of the cuvette with the CE of the potentiostat.
   e. Connecting the RE of the cuvette with the RE of the potentiostat.
   f. Connecting the WE of the cuvette with the WE of the potentiostat.
   g. Connecting the ground of the electrode wires with the ground of the potentiostat.

1.8 – Chemical Resistance.

The material of the pump, the cuvette, the disk/chip and the Teflon tubing determine the chemical resistance of the instrument. Aqueous buffer solution without organic compounds can be used without damaging the sample handling part of the instrument (Table 1.7.1 and 1.7.2). Some organic solvents are not recommended for use in the TWINGEL/SPRINGLE instrument, they may damage the system (Table 1.7.3).

Table 1.7.1: Recommended buffer solutions.

Solution	Concentration	pH	Solution	Concentration	pH
ACES	50 mM	6.8	MES	50 mM	6.1
ADA	50 mM	6.6	MOPS	50 mM	7.2
BES	50 mM	7.1	MOPSO	50 mM	6.9
BICINE	50 mM	8.3	Phosphate	50 mM	7.5
BIS-TRIS	50 mM	6.5	PIPES	50 mM	6.8
Borate	100 mM	8.8	POPSO	50 mM	7.8
CAPS	50 mM	10.4	TAPS	50 mM	8.4
CHES	50 mM	9.3	TED	50 mM	7.5
Citrate	50 mM	3.0	TRICINE	50 mM	8.1
EPPS	50 mM	8.0	TRIS-HCl	75 mM	8.0
Glycine	50 mM	2.3	TRIZMA BASE	50 mM	8.1
HEPES	50 mM	7.5			

Table 1.7.2: Recommended regeneration solutions.

Solution	Concentration	pH
Acetonitrile	20%	7.5
Hydrochloric acid	10 - 1000 mM	
Ethanol	10 - 100 %	
Formic acid	1 - 20 %	
Glycine	0.01- 2 M	2.5 -3.5
Phosphoric acid	0 - 1000 mM	
SDS	5 - 20 %	
Sodium carbonate	200 mM	11.5
Sodium chloride	1000 mM	
Sodium hydroxide	10 - 1000 mM	

Table 1.7.3: Organic solvents NOT recommended for the Autolab TWINGLE instrument.

Solution	Concentration	Solution	Concentration
Acetone	100 %	Ethyl chromide	100 %
Amyl acetate	100 %	Methyl Ethyl Ketone	100 %
Benzene	100 %	Methylene Chloride	100 %
Butyl alcohol	100 %	Nitric Acid	100 %
Carbon tetrachloride	100 %	Pyridine	100 %
Chlorine	100 %	Sulphuric Acid	100 %
Chloroform	100 %	Toluene	100 %
Chromic acid	100 %	Trichloroethylene	100 %
Cyclohexane	100 %	Xylene	100 %
Ethyl acetate	100 %		

1.9 – Materials.

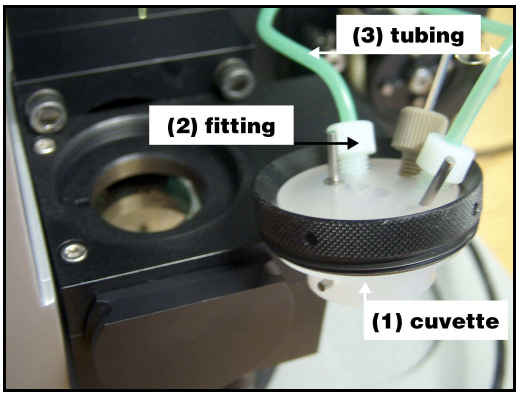


Figure 1.4 – Cuvette, tubing and fitting.

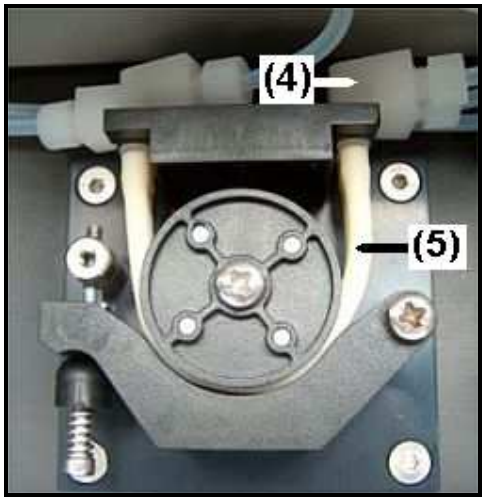


Figure 1.5 – Peristaltic pump.

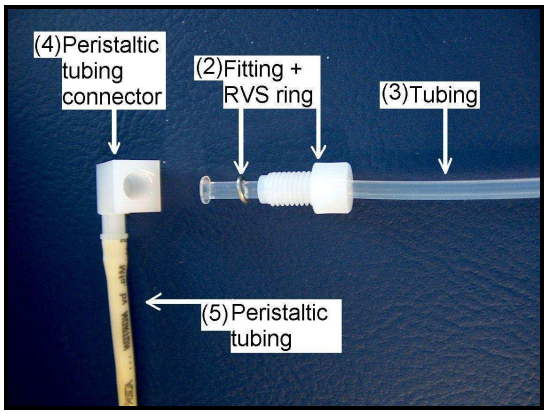


Figure 1.6 – Peristaltic pump tubing.

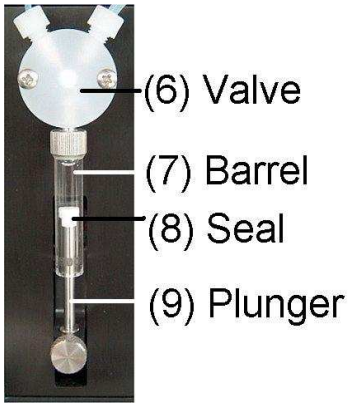


Figure 1.7 – Syringe pump.

Item;	Material;
1 = Cuvette	standard KEL-F / special PVDF
2 = Fitting	Tefzel (ETFE)
3 = Tubing	Teflon
4 = Connector peristaltic tubing	PVDF
5 = Peristaltic pump tubing	Pharmed
6 = Valve	Kel-F
7 = Syringe barrel	Borosilicate glass
8 = Syringe seal	Teflon
9 = Syringe plunger	Stainless steel, RVS

- PVDF
  Polyvinyllidene Fluoride. Excellent chemical resistance. Ideal for the cuvette and tubing connections.
- KEL-F
  PCTFE (polychloro-trifluoroethylene). Excellent chemical resistance, ideal for fittings and sealing surfaces. THF (tetrahydrofuran) and a few halogenated solvents will react with it.
- PEEK
  Polyetheretherketone. Excellent chemical resistance, although not recommended with nitric acid, sulphuric acid, halogenated acids and pure halogenated gases. Also, a swelling effect occurs with methylene chloride, THF, and DMSO.
- Teflon FEP and PFA
  Fluorinated ethylene propylene and perfluoroalkoxy alkane. Inert to virtually all chemicals.
- Tefzel ETFE
  Ethylene-tetrafluoroethylene. Excellent solvent resistance.

	Kel-F	PEEk	PVDF	FEP/PFA	Tefzel
Solvent					
Aromatics	R	R	R	R	R
Chlorinated	M	M	R	R	R
Ketones	R	R	R	R	R
Aldehydes	R	R	R	R	R
Ethers	M	M	R	R	R
Amines	R	R	R	R	M
Aliphatic sol.	R	R	R	R	R
Organic Acids	R	M	R	R	R
Inorganic Acids	R	M	R	R	M
Bases	R	R	R	R	R
Sulfonated Compounds	R	M	R	R	R
Thread strength	Good	Excellent	Excellent	Good	Good

R = Recommended; NR = Not recommended; M = Moderate resistance

Chapter 2

2 – Software Installation.

2.1 – Index.

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2.2 – Introduction.

The Autolab SPR installation CD contains the Autolab ESPRIT, TWINGLE and SPRINGLE instruments software. During the software installation, choose the right setup software for the corresponding Autolab SPR instrument. The software can be used on the Windows 2000/XP or Vista platforms. Insert the CD into the computer and the installation will start automatically (see Figure 2.1).

2.3 – Installation of the Autolab SPR software.

Start the computer and wait until the Windows start-up is finished:
- Close all open programs,
- Insert the Data acquisition installation software CD in the CD-drive,
- Wait until the automatic setup screen appears.

IF the automatic 'Setup' screen does not appear after inserting the CD:
- Select "Run.." from the windows Start menu,
- Browse to the Autolab SPR CD-ROM,
- Open "SETUP.EXE",
- Select OK in the "RUN" screen.

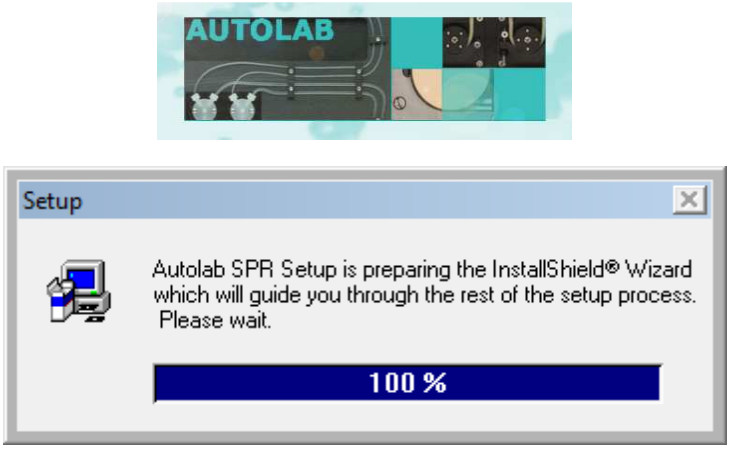

AUTOLAB
Setup
Autolab SPR Setup is preparing the InstallShield® Wizard which will guide you through the rest of the setup process. Please wait.
100 %

Figure 2.1 – Start of the Setup procedure.

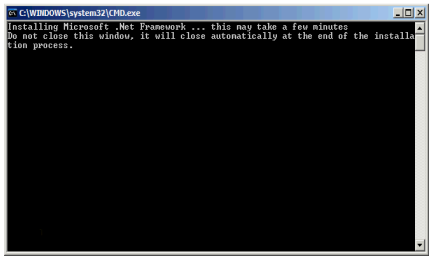

C:\WINDOWS\system32\CMD.exe
Installing Microsoft .Net Framework ... this may take a few minutes
Do not close this window, it will close automatically at the end of the installa
tion process.

Figure 2.2 – Installation window 2.
This window appears for a very short time and disappears automatically

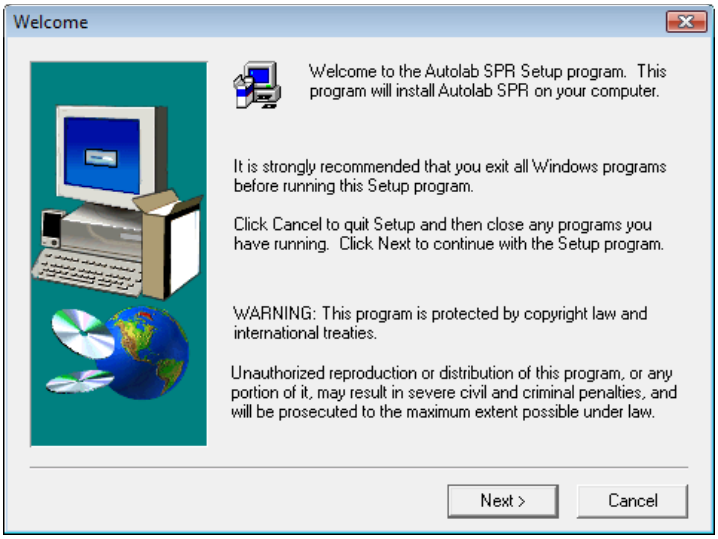

Welcome
Welcome to the Autolab SPR Setup program. This program will install Autolab SPR on your computer.
It is strongly recommended that you exit all Windows programs before running this Setup program.
Click Cancel to quit Setup and then close any programs you have running. Click Next to continue with the Setup program.
WARNING: This program is protected by copyright law and international treaties.
Unauthorized reproduction or distribution of this program, or any portion of it, may result in severe civil and criminal penalties, and will be prosecuted to the maximum extent possible under law.
Next >
Cancel

Figure 2.3 – Installation window 3, 'Welcome.'
Press 'Next' to proceed with the installation procedure.

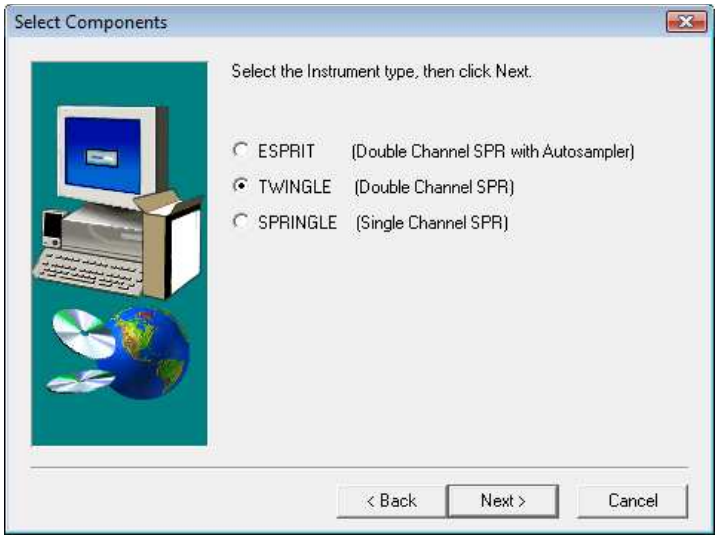

Select Components
Select the Instrument type, then click Next.
ESPRIT (Double Channel SPR with Autosampler)
TWINGLE (Double Channel SPR)
SPRINGLE (Single Channel SPR)
< Back
Next >
Cancel

Figure 2.4 – Installation window 4 for Autolab TWINGLE.
Press 'Next' to proceed with the installation procedure.

Select the Autolab SPR instrument type TWINGLE which the software should be in command of.

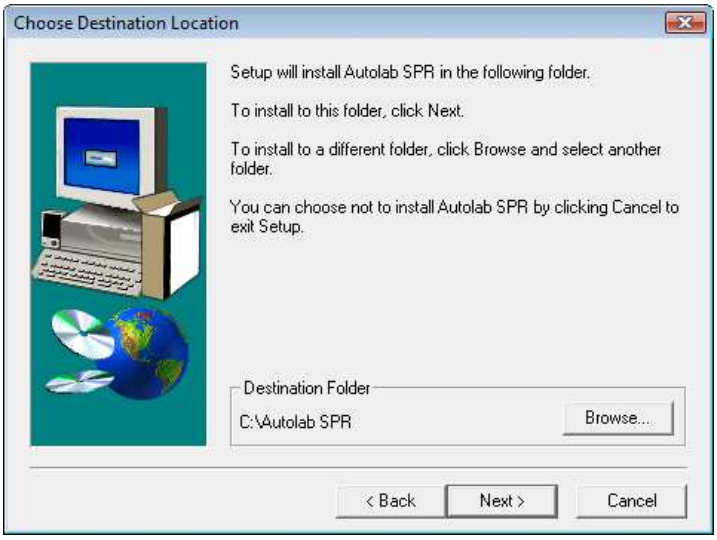

Choose Destination Location
Setup will install Autolab SPR in the following folder.
To install to this folder, click Next.
To install to a different folder, click Browse and select another folder.
You can choose not to install Autolab SPR by clicking Cancel to exit Setup.
Destination Folder
C:\Autolab SPR
Browse...
< Back
Next >
Cancel

Figure 2.5 – Installation window 5.
Press 'Next' to proceed with the installation procedure.

A. Autolab SPR or rename B. Autolab TWINGLE

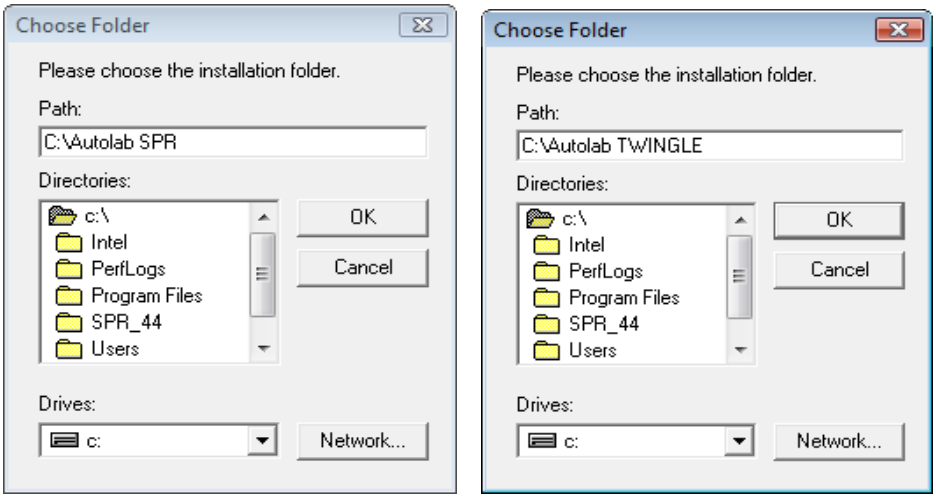


Figure 2.6 – Installation window 6 Twingle.
Not necessary, but the name of the destination folder can be changed. So, for the SPRINGLE installation the name can be changed in SPRINGLE. (Again it is not necessary!). Press 'OK' to confirm the new folder name and location.

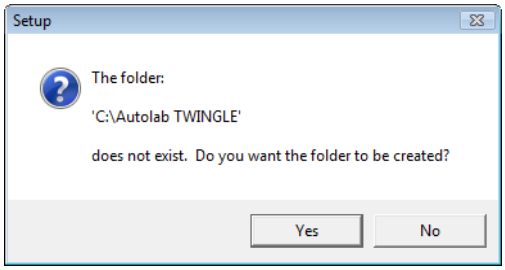
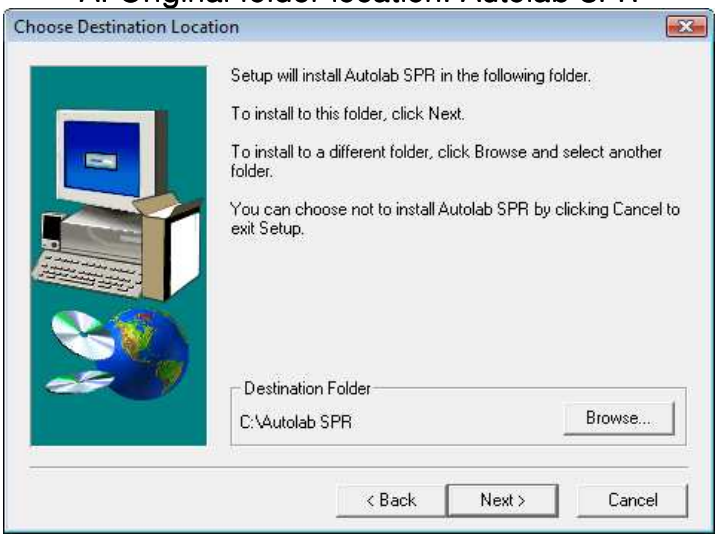


Figure 2.7 – Installation window 7.

When the folder does not exist press 'Yes' to create the folder. If the folder name has been changed into Autolab SPRINGLE, this box would ask for confirmation as well.

A. Original folder location: Autolab SPR


Choose Destination Location
Setup will install Autolab SPR in the following folder.
To install to this folder, click Next.
To install to a different folder, click Browse and select another folder.
You can choose not to install Autolab SPR by clicking Cancel to exit Setup.
Destination Folder
C:\Autolab SPR
Browse...
< Back
Next >
Cancel

B. New folder location: Autolab TWINGLE

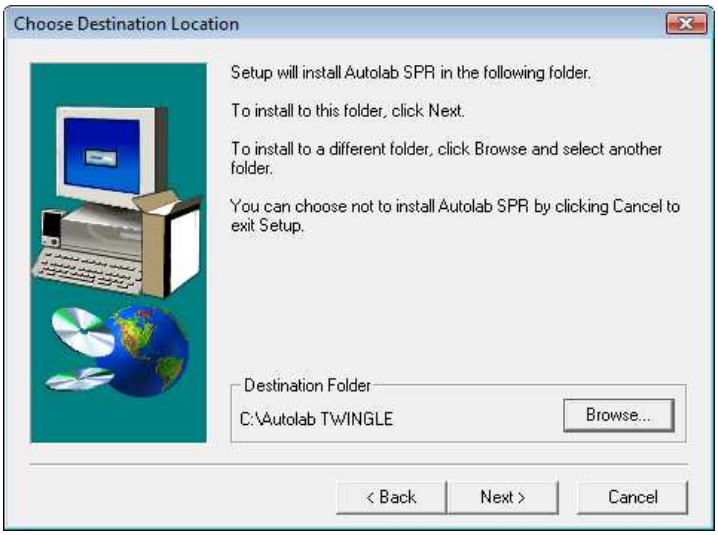
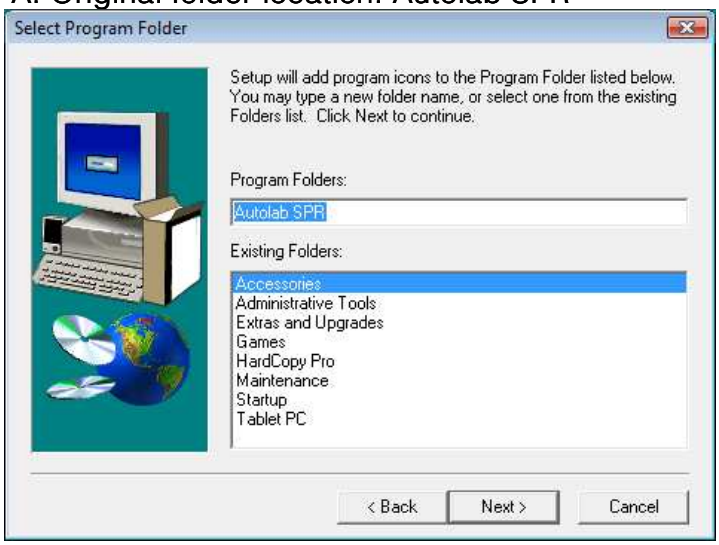

Choose Destination Location
Setup will install Autolab SPR in the following folder.
To install to this folder, click Next.
To install to a different folder, click Browse and select another folder.
You can choose not to install Autolab SPR by clicking Cancel to exit Setup.
Destination Folder
C:\Autolab TWINGLE
Browse...
< Back
Next >
Cancel

Figure 2.8 – Installation window 8.

Press 'Next' to proceed with the installation procedure.

A. Original folder location: Autolab SPR



B. New folder location: Autolab TWINGLE

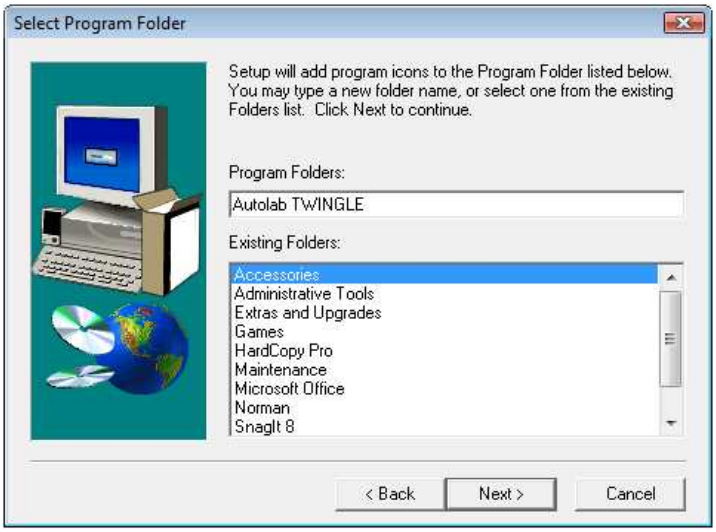


Figure 2.9 – Installation window 9.

The program folder name can be changed in TWINGLE or SPRINGLE as well, like in picture B.

Press 'Next' to proceed with the installation procedure.

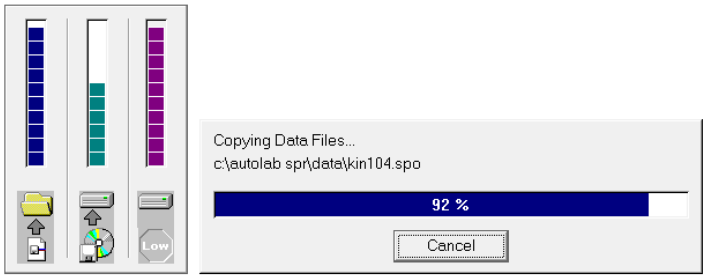


Figure 2.10 – Installation window 10.

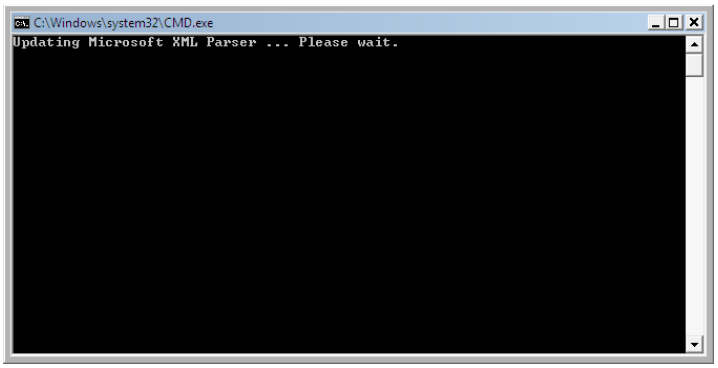


Figure 2.11 – Installation window 11.
This screen pops up for just a very short moment.

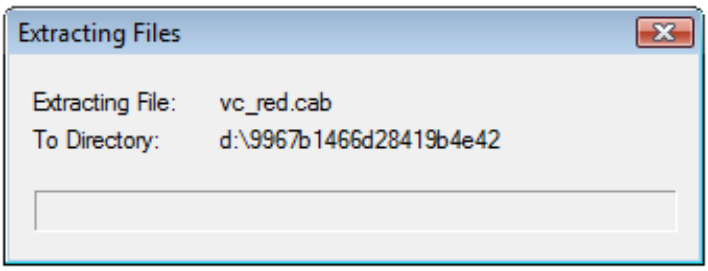


Figure 2.12 – Installation window 12.
This screen pops up for just a very short moment.

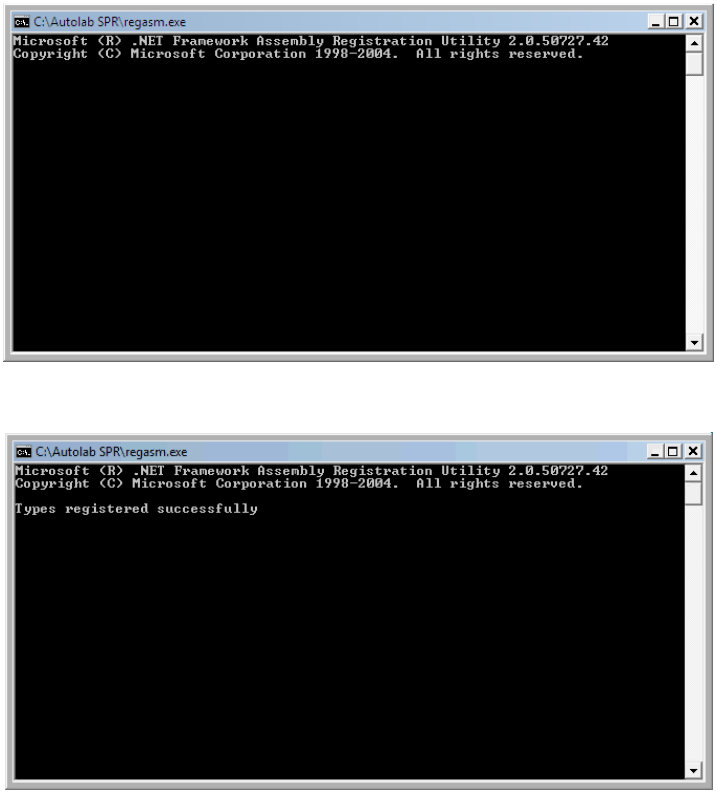
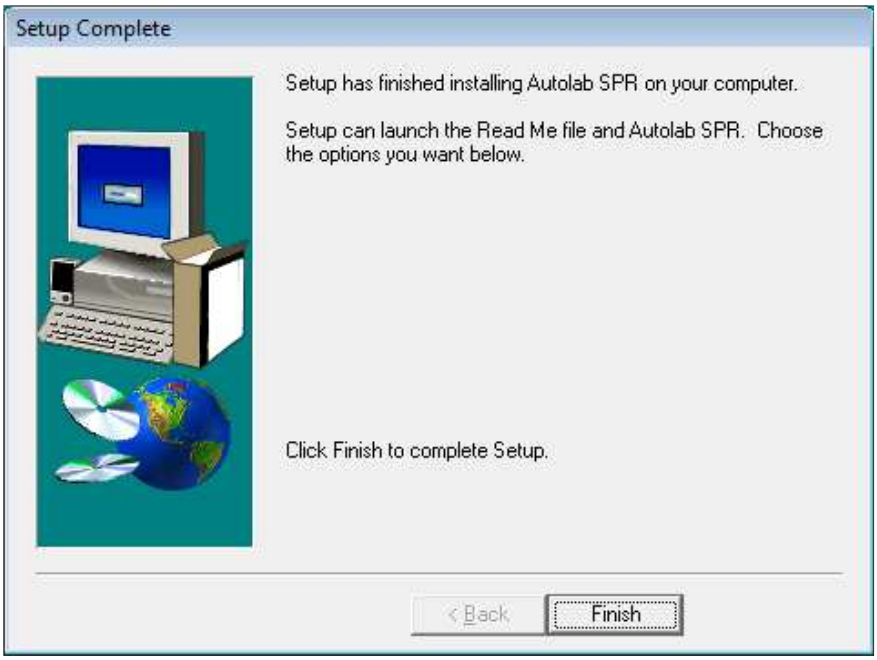

C:\Autolab SPR\regasm.exe
Microsoft (R) .NET Framework Assembly Registration Utility 2.0.50727.42
Copyright (C) Microsoft Corporation 1998-2004. All rights reserved.
C:\Autolab SPR\regasm.exe
Microsoft (R) .NET Framework Assembly Registration Utility 2.0.50727.42
Copyright (C) Microsoft Corporation 1998-2004. All rights reserved.
Types registered successfully

Figure 2.13 – Installation window 13.
These two screens pop up at different positions a few times and disappear again very quickly.


Setup Complete
Setup has finished installing Autolab SPR on your computer.
Setup can launch the Read Me file and Autolab SPR. Choose the options you want below.
Click Finish to complete Setup.
< Back
Finish

A shortcut icon to the Data Acquisition program and the kinetic evaluation program will be installed on your desktop (figure 2.15).

Fig 2.15 – The desktop icons shown after the installation of the SPR software.

2.4 – Autolab SPR software setup on the hard disk after installation.

2.4.1 – Folder structure.

The Autolab SPR folder is created during the installation of the SPR software. There are two folders for sequences in the Twingle software. The main sequence folder filled with sequences to use and one sub-folder for include sequences (see chapter 6). The Autolab SPR root folder contains the executable for the Kinetic Evaluation software.

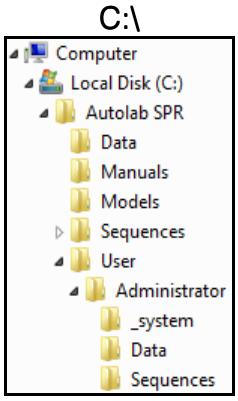
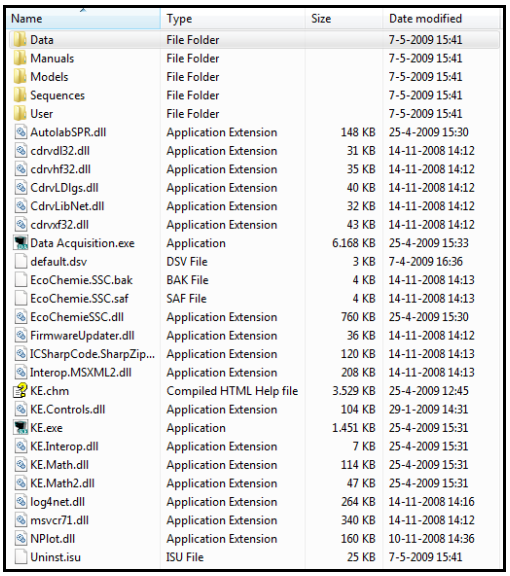


Figure 2.16 – Folder structure.

Fig 2.16; Shows the Autolab SPR folder structure; C:\Autolab SPR\ with the TWINGLE/SPRINGLE installation. The subdirectory “USER” is only installed in the Good Laboratory Practice software version.

2.4.2 – Files in C:\Autolab SPR\.

Files in the root of the Autolab SPR folder are shown below. The “user” folder will only be installed with the security software version.

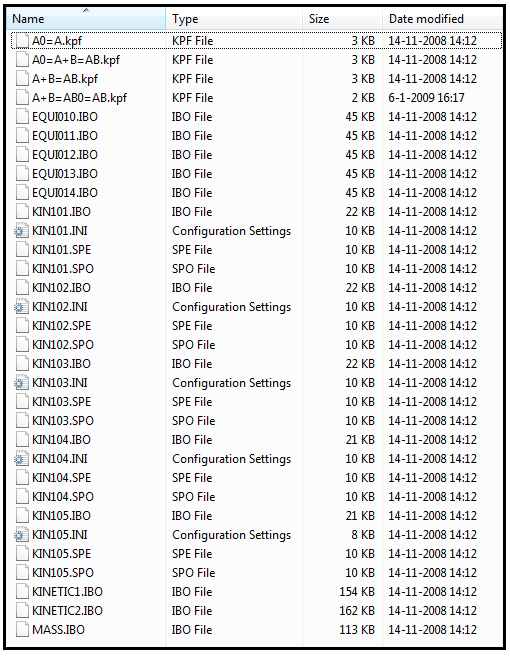


Name	Type	Size	Date modified
Data	File Folder		7-5-2009 15:41
Manuals	File Folder		7-5-2009 15:41
Models	File Folder		7-5-2009 15:41
Sequences	File Folder		7-5-2009 15:41
User	File Folder		7-5-2009 15:41
AutolabSPR.dll	Application Extension	148 KB	25-4-2009 15:30
cdrvdl32.dll	Application Extension	31 KB	14-11-2008 14:12
cdrvhf32.dll	Application Extension	35 KB	14-11-2008 14:12
CdrvLDlgs.dll	Application Extension	40 KB	14-11-2008 14:12
CdrvLibNet.dll	Application Extension	32 KB	14-11-2008 14:12
cdrvxf32.dll	Application Extension	43 KB	14-11-2008 14:12
Data Acquisition.exe	Application	6.168 KB	25-4-2009 15:33
default.dsv	DSV File	3 KB	7-4-2009 16:36
EcoChemie.SSC.bak	BAK File	4 KB	14-11-2008 14:13
EcoChemie.SSC.saf	SAF File	4 KB	14-11-2008 14:13
EcoChemieSSC.dll	Application Extension	760 KB	25-4-2009 15:30
FirmwareUpdater.dll	Application Extension	36 KB	14-11-2008 14:12
ICSharpCode.SharpZip...	Application Extension	120 KB	14-11-2008 14:13
Interop.MSXML2.dll	Application Extension	208 KB	14-11-2008 14:13
KE.chm	Compiled HTML Help file	3.529 KB	25-4-2009 12:45
KE.Controls.dll	Application Extension	104 KB	29-1-2009 14:31
KE.exe	Application	1.451 KB	25-4-2009 15:31
KE.Interop.dll	Application Extension	7 KB	25-4-2009 15:31
KE.Math.dll	Application Extension	114 KB	25-4-2009 15:31
KE.Math2.dll	Application Extension	47 KB	25-4-2009 15:31
log4net.dll	Application Extension	264 KB	14-11-2008 14:16
msvcr71.dll	Application Extension	340 KB	14-11-2008 14:12
NPlot.dll	Application Extension	160 KB	10-11-2008 14:36
Uninst.isu	ISU File	25 KB	7-5-2009 15:41

Figure 2.17 – Content of C:\Autolab SPR.. folder.

2.4.3 – Examples of data files in the subfolder C:\Autolab SPR\Data.

The shown files are original experimental data. One measurement has four different saved files with different extensions; *.IBO, *.SPE, *.SPO, *.INI extension has all experimental data. The current software will save measured SPR data in just one file with the extension *.SPR.



Name	Type	Size	Date modified
A0=A.kpf	KPF File	3 KB	14-11-2008 14:12
A0=A+B=AB.kpf	KPF File	3 KB	14-11-2008 14:12
A+B=AB.kpf	KPF File	3 KB	14-11-2008 14:12
A+B=AB0=AB.kpf	KPF File	2 KB	6-1-2009 16:17
EQUI010.IBO	IBO File	45 KB	14-11-2008 14:12
EQUI011.IBO	IBO File	45 KB	14-11-2008 14:12
EQUI012.IBO	IBO File	45 KB	14-11-2008 14:12
EQUI013.IBO	IBO File	45 KB	14-11-2008 14:12
EQUI014.IBO	IBO File	45 KB	14-11-2008 14:12
KIN101.IBO	IBO File	22 KB	14-11-2008 14:12
KIN101.INI	Configuration Settings	10 KB	14-11-2008 14:12
KIN101.SPE	SPE File	10 KB	14-11-2008 14:12
KIN101.SPO	SPO File	10 KB	14-11-2008 14:12
KIN102.IBO	IBO File	22 KB	14-11-2008 14:12
KIN102.INI	Configuration Settings	10 KB	14-11-2008 14:12
KIN102.SPE	SPE File	10 KB	14-11-2008 14:12
KIN102.SPO	SPO File	10 KB	14-11-2008 14:12
KIN103.IBO	IBO File	22 KB	14-11-2008 14:12
KIN103.INI	Configuration Settings	10 KB	14-11-2008 14:12
KIN103.SPE	SPE File	10 KB	14-11-2008 14:12
KIN103.SPO	SPO File	10 KB	14-11-2008 14:12
KIN104.IBO	IBO File	21 KB	14-11-2008 14:12
KIN104.INI	Configuration Settings	10 KB	14-11-2008 14:12
KIN104.SPE	SPE File	10 KB	14-11-2008 14:12
KIN104.SPO	SPO File	10 KB	14-11-2008 14:12
KIN105.IBO	IBO File	21 KB	14-11-2008 14:12
KIN105.INI	Configuration Settings	8 KB	14-11-2008 14:12
KIN105.SPE	SPE File	10 KB	14-11-2008 14:12
KIN105.SPO	SPO File	10 KB	14-11-2008 14:12
KINETIC1.IBO	IBO File	154 KB	14-11-2008 14:12
KINETIC2.IBO	IBO File	162 KB	14-11-2008 14:12
MASS.IBO	IBO File	113 KB	14-11-2008 14:12

Figure 2.18 – Content of C:\Autolab SPR\Data.. folder.

2.4.4 – Softcopy manuals in the subdirectory MANUALS.

C:\Autolab SPR\Manuals

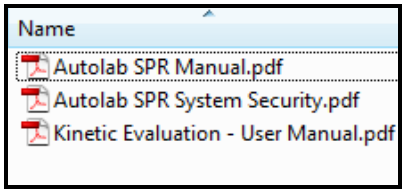


Figure 2.19 – Manuals installed during the installation of the software.
The Autolab SPR System Security manual will only be installed if the Security version has been installed.

2.4.5 – Examples of KE models in the subdirectory MODELS.

C:\Autolab SPR\Models

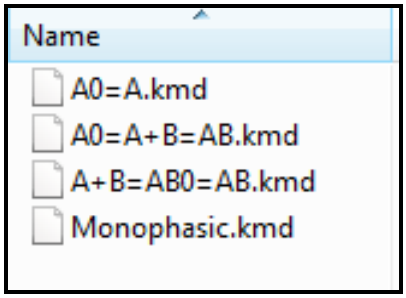


Figure 2.20 – Examples of kinetic evaluation models installed with the software.

2.4.6 – Examples of KE PROJECT files in the subdirectory Data.

C:\Autolab SPR\Data

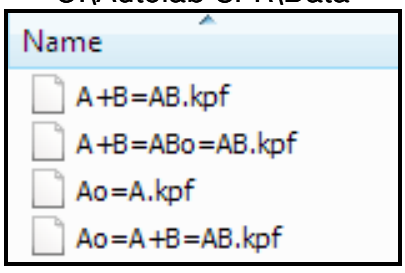


Figure 2.21 – Examples of kinetic evaluation projects installed with the software.

2.4.7 – Autolab TWINGLE sequence files in subdirectory SEQUENCES.

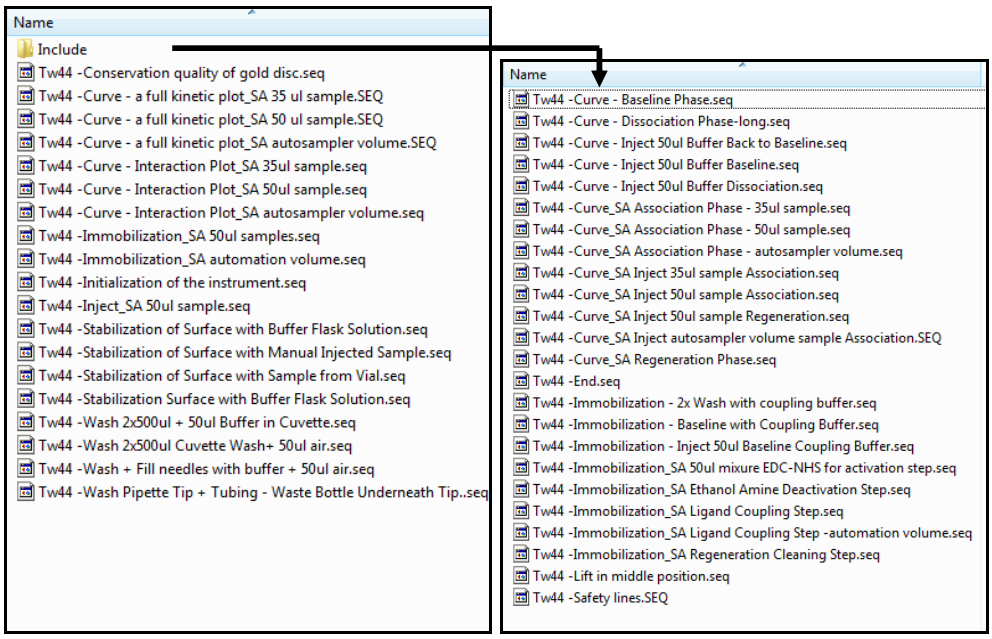

Name
Include
Tw44 -Conservation quality of gold disc.seq
Tw44 -Curve - a full kinetic plot_SA 35 ul sample.SEQ
Tw44 -Curve - a full kinetic plot_SA 50 ul sample.SEQ
Tw44 -Curve - a full kinetic plot_SA autosampler volume.SEQ
Tw44 -Curve - Interaction Plot_SA 35ul sample.seq
Tw44 -Curve - Interaction Plot_SA 50ul sample.seq
Tw44 -Curve - Interaction Plot_SA autosampler volume.seq
Tw44 -Immobilization_SA 50ul samples.seq
Tw44 -Immobilization_SA automation volume.seq
Tw44 -Initialization of the instrument.seq
Tw44 -Inject_SA 50ul sample.seq
Tw44 -Stabilization of Surface with Buffer Flask Solution.seq
Tw44 -Stabilization of Surface with Manual Injected Sample.seq
Tw44 -Stabilization of Surface with Sample from Vial.seq
Tw44 -Stabilization Surface with Buffer Flask Solution.seq
Tw44 -Wash 2x500ul + 50ul Buffer in Cuvette.seq
Tw44 -Wash 2x500ul Cuvette Wash+ 50ul air.seq
Tw44 -Wash + Fill needles with buffer + 50ul air.seq
Tw44 -Wash Pipette Tip + Tubing - Waste Bottle Underneath Tip..seq
Name
Tw44 -Curve - Baseline Phase.seq
Tw44 -Curve - Dissociation Phase-long.seq
Tw44 -Curve - Inject 50ul Buffer Back to Baseline.seq
Tw44 -Curve - Inject 50ul Buffer Baseline.seq
Tw44 -Curve - Inject 50ul Buffer Dissociation.seq
Tw44 -Curve_SA Association Phase - 35ul sample.seq
Tw44 -Curve_SA Association Phase - 50ul sample.seq
Tw44 -Curve_SA Association Phase - autosampler volume.seq
Tw44 -Curve_SA Inject 35ul sample Association.seq
Tw44 -Curve_SA Inject 50ul sample Association.seq
Tw44 -Curve_SA Inject 50ul sample Regeneration.seq
Tw44 -Curve_SA Inject autosampler volume sample Association.SEQ
Tw44 -Curve_SA Regeneration Phase.seq
Tw44 -End.seq
Tw44 -Immobilization - 2x Wash with coupling buffer.seq
Tw44 -Immobilization - Baseline with Coupling Buffer.seq
Tw44 -Immobilization - Inject 50ul Baseline Coupling Buffer.seq
Tw44 -Immobilization_SA 50ul mixure EDC-NHS for activation step.seq
Tw44 -Immobilization_SA Ethanol Amine Deactivation Step.seq
Tw44 -Immobilization_SA Ligand Coupling Step.seq
Tw44 -Immobilization_SA Ligand Coupling Step -automation volume.seq
Tw44 -Immobilization_SA Regeneration Cleaning Step.seq
Tw44 -Lift in middle position.seq
Tw44 -Safety lines.SEQ

Figure 2.22 – Sequences for the Autolab TWINGLE instrument.

Figure 2.22 shows typical Autolab TWINGLE sequences to perform double channel SPR experiments. The left picture has all stand alone sequences for specific experiments. The sequences in the right picture are components to create sequences as shown in the left picture and are called “Include Sequences”. More details about reading, adjusting and creating these sequences can be found in Chapter 6.

2.4.8 – Autolab SPRINGLE sequence files in subdirectory SEQUENCES.

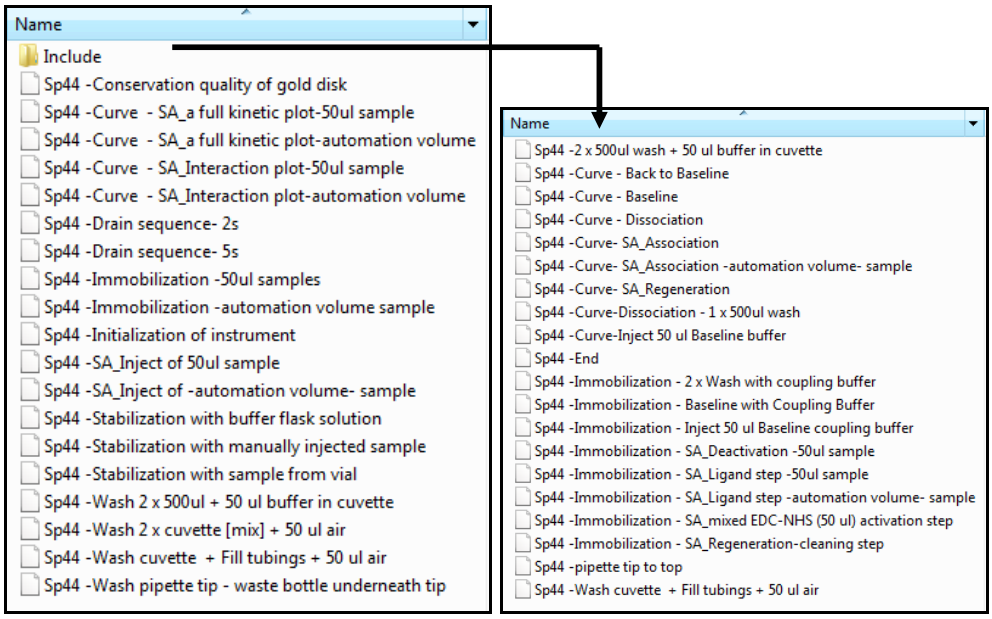

Name
Include
Sp44 -Conservation quality of gold disk
Sp44 -Curve - SA_a full kinetic plot-50ul sample
Sp44 -Curve - SA_a full kinetic plot-automation volume
Sp44 -Curve - SA_Interaction plot-50ul sample
Sp44 -Curve - SA_Interaction plot-automation volume
Sp44 -Drain sequence- 2s
Sp44 -Drain sequence- 5s
Sp44 -Immobilization -50ul samples
Sp44 -Immobilization -automation volume sample
Sp44 -Initialization of instrument
Sp44 -SA_Inject of 50ul sample
Sp44 -SA_Inject of -automation volume- sample
Sp44 -Stabilization with buffer flask solution
Sp44 -Stabilization with manually injected sample
Sp44 -Stabilization with sample from vial
Sp44 -Wash 2 x 500ul + 50 ul buffer in cuvette
Sp44 -Wash 2 x cuvette [mix] + 50 ul air
Sp44 -Wash cuvette + Fill tubings + 50 ul air
Sp44 -Wash pipette tip - waste bottle underneath tip
Name
Sp44 -2 x 500ul wash + 50 ul buffer in cuvette
Sp44 -Curve - Back to Baseline
Sp44 -Curve - Baseline
Sp44 -Curve - Dissociation
Sp44 -Curve- SA_Association
Sp44 -Curve- SA_Association -automation volume- sample
Sp44 -Curve- SA_Regeneration
Sp44 -Curve-Dissociation - 1 x 500ul wash
Sp44 -Curve-Inject 50 ul Baseline buffer
Sp44 -End
Sp44 -Immobilization - 2 x Wash with coupling buffer
Sp44 -Immobilization - Baseline with Coupling Buffer
Sp44 -Immobilization - Inject 50 ul Baseline coupling buffer
Sp44 -Immobilization - SA_Deactivation -50ul sample
Sp44 -Immobilization - SA_Ligand step -50ul sample
Sp44 -Immobilization - SA_Ligand step -automation volume- sample
Sp44 -Immobilization - SA_mixed EDC-NHS (50 ul) activation step
Sp44 -Immobilization - SA_Regeneration-cleaning step
Sp44 -pipette tip to top
Sp44 -Wash cuvette + Fill tubings + 50 ul air

Figure 2.23 – Sequences for the Autolab SPRINGLE instrument.

Figure 2.23 shows typical Autolab SPRINGLE sequences to perform double channel SPR experiments. The left picture has all stand alone sequences for specific experiments. The sequences in the right picture are components to create sequences as shown in the left picture and are called “Include Sequences”. More details about reading, adjusting and creating these sequences can be found in Chapter 6.

Chapter 3

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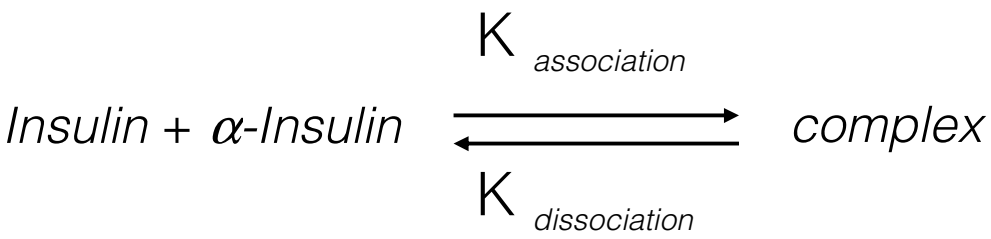
3.2 – Introduction.

This "getting started" document will take you step by step through the initialization of the TWINGLE/SPRINGLE instrument and, subsequently, through an interaction experiment where the antibody anti-Insuline will be interacting with the immobilized protein Insulin. Throughout this procedure, most features of the software will be illustrated.

It is presumed that the hardware and software have been installed before; the cuvette and hemi-cylinder are disassembled and all tubing is empty. A 60 min. warm-up time of the Autolab TWINGLE should be taken into account.

Detection of binding events between Insulin and anti-Insulin



First, the modified gold layer on the sensor disk is coated with mercapto-undecanoic acid (11-MUA). After being assembled into the instrument, the modified surface is stabilized with the immobilization coupling buffer. In the immobilization procedure (Immobilization), the acid group of this molecule is activated by incubation with EDC and NHS. Subsequently, the Insulin is immobilized onto the modified gold layer.
The Insulin layer will thereafter be stabilized with association buffer. In the interaction phase (Interaction), several dilutions of anti-Insulin are used to visualize the Insulin / anti-Insulin interaction. In the reference channel the effect of the plain association buffer is recorded to correct for any a-specific interaction factors. After the association phase, which is used to calculate the association constant Ka, the dissociation phase is performed by washing the sample away with association buffer. The association buffer is the same buffer as the dilution buffer for the anti-Insulin. The dissociation phase is used for determining the strength of the interaction. After the dissociation phase, all bound anti-Insulin is removed from the Insulin coated gold disk by diluted SDS, Sodium dodecyl sulphate, (regeneration buffer). Then, the baseline of the modified disk will be restored by washing the regeneration solution replacing it with the baseline HEPES buffer.

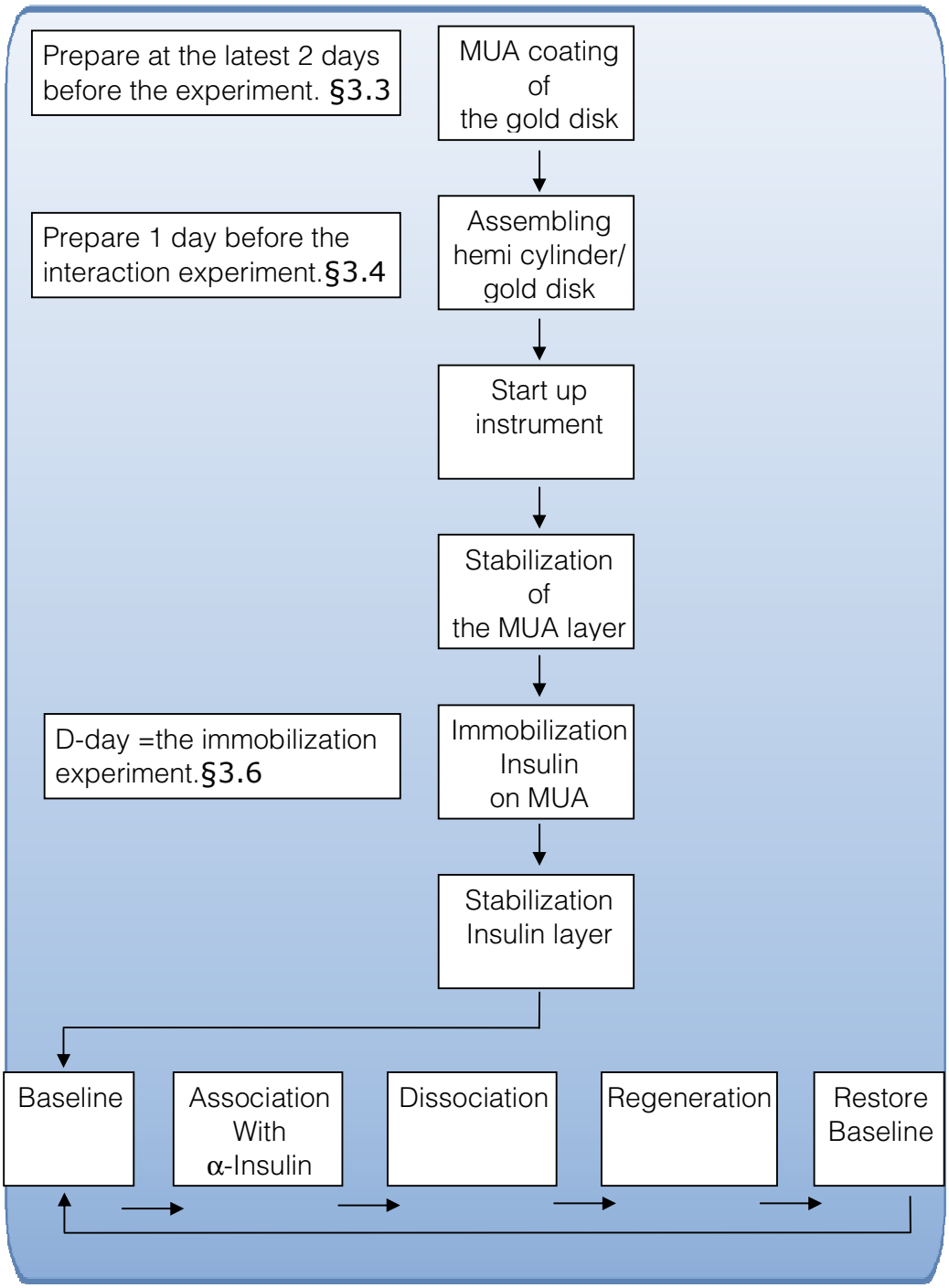


Figure 3.1 – Flow chart of the experimental setup.

After one initial immobilization, numerous SPR experiments can be performed. The surface plasmon resonance (SPR) measures angle versus time. There is a linear relationship between the amount of bound material and shift in SPR angle. The SPR angle shift, in millidegrees (m°), is used as a response unit to quantify the binding of macromolecules to the sensor surface. A change of 122 m° represents a change in surface protein mass of approximately 1 ng/mm^2.

3.3 – Two days before the experiment.

3.3.1 – Gold surface modification with a 11-MUA layer.

Chemicals: 11 – Mercapto-undecanoic acid (11-MUA), Aldrich 450561,

Preparation: 1 mM 11-Mercaptoundecanoic acid (11-MUA):
Dissolve 11 mg 11-Mercaptoundecanoic acid (Mw. 218.36) in 50 ml alcohol; like methanol, ethanol or (iso)propanol.

Incubate a bare gold disk in a 6 well culture disk in a solution of 11mg 11-MUA in 50 ml alcohol. This molecule will self assemble a monolayer onto the gold surface. To get a reproducible quality thiol layer, filter the solution prior to the incubation and perform the incubation overnight.

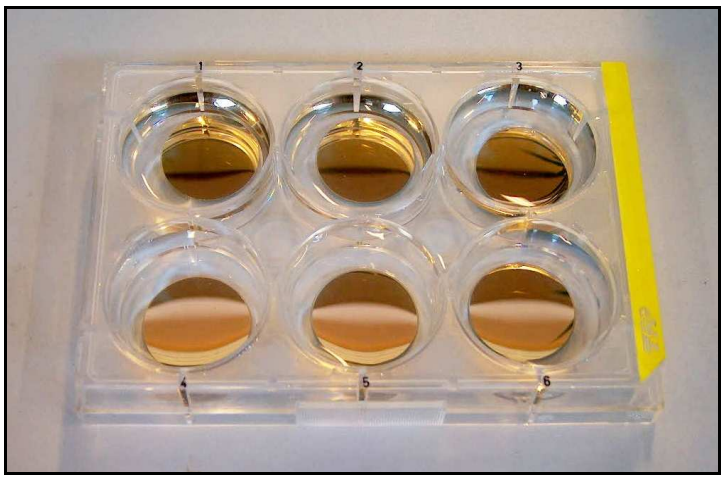


Figure 3.2 – 6 Well Microtiter culture plate.

3.4 – One day before the experiment.

3.4.1 – Washing the 11-MUA modified gold surface.

Wash the disk three times with alcohol to remove the excess thiol. To remove the alcohol, rinse three times with demineralized water. Blow the disk dry with compressed air or nitrogen gas. The thiol covered gold disk can be stored dry up to 2 months in the original container.

3.4.2 – Startup of the Autolab instrument.

- Install the power supply cable and connect the com port of the instrument to the computer with the RS232 connector cable.
- Switch on the Autolab instrument. Use the main switch on the back panel (Figure 1.1) and the power button situated at the right top

position of the front panel. The LED in the power button lights up after a few seconds.

- Start the Autolab SPR Data Acquisition software. Wait until the instrument has finished initiating the TWINGLE/SPRINGLE “lift” and the syringe pumps, this will take about 20 seconds.
- A warm-up time of about 1 hour should be taken into account before measuring with the Autolab TWINGLE/SPRINGLE.

Figure 3.3 – the back panel of the TWINGLE.
Left side the black power switch I/O.

3.4.3 – Liquid Handling set up Autolab.

Before the system can be used, all tubing needs to be filled with buffer. Fill buffer flask with HEPES buffer and insert the inlet tubing of the syringe pump into the running buffer flask and the green outlet tubing of the peristaltic ‘drain’ pump into the waste bottle.

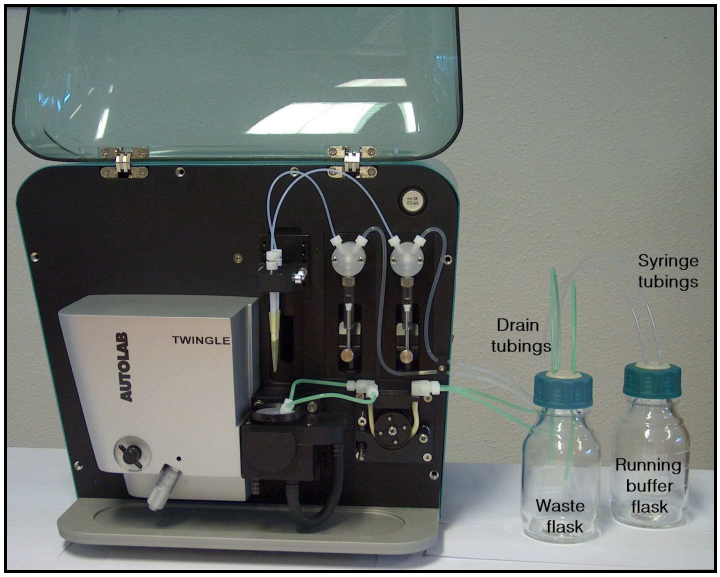


Figure 3.4 – The draining tube from the drain peristaltic pump is inserted into the waste bottle (green tubing in reality). Both syringe tubes are inserted into the buffer flask (SPRINGLE has only ONE syringe tubing.).

3.4.4 – Initialization of the instrument.

Before using the instrument:

- Calibrate lift positions.
- Assemble a new sensor disk
- Assemble the cuvette
- Fill the tubing with running buffer solution

3.4.5 – Autolab lift position calibration.

There are three ways to find out if the lift is calibrated;

- In the menu TWINGLE/SPRINGLE find the 'lift position' item to check the positions, up, middle, down,
- In the menu TWINGLE/SPRINGLE find the 'Manual control' item to check the three positions,
- In the toolbar find the button 'lift positions'

Open the 'Manual Control' window in the 'TWINGLE/SPRINGLE' Menu or with the button in the toolbar.

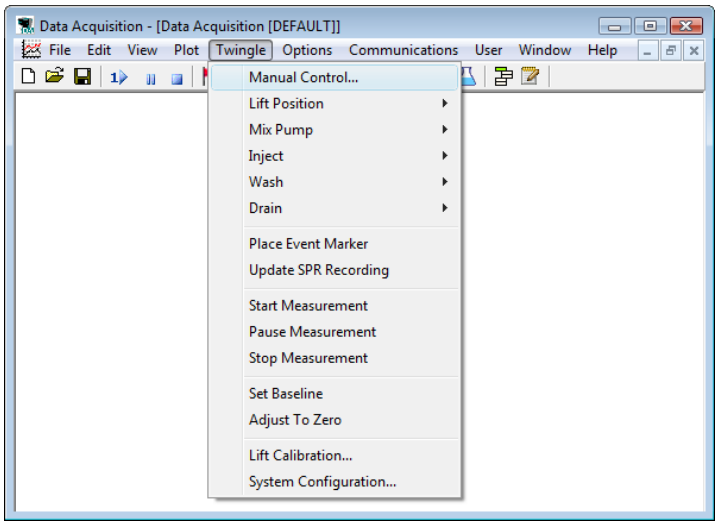


Figure 3.5 – Menu TWINGLE to open 'Manual Control' window.

The lift position has to be calibrated every time you change the pipette tip.

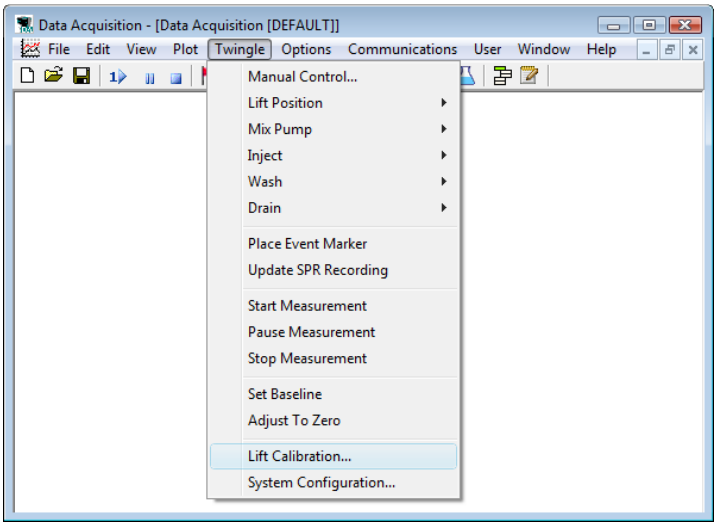


Figure 3.6 – Open the Lift calibration window.

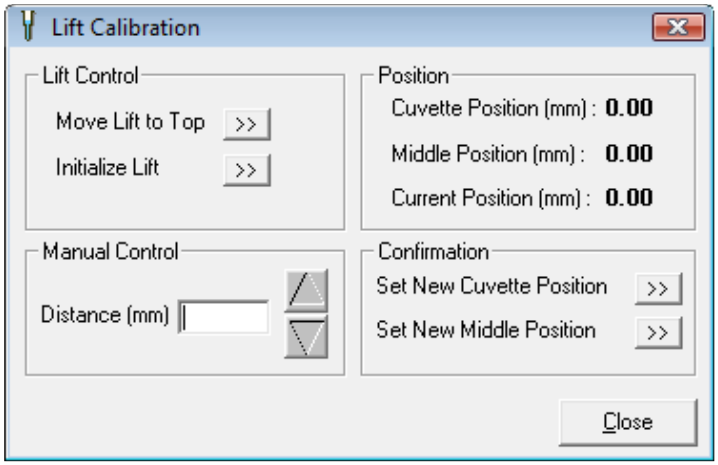


Figure 3.7 – The lift calibration window.

The lift calibration sets the pipette position. The cuvette position for the pipette is normally calibrated to 1 mm above the gold disk; the middle position is calibrated with the pipette just about 2 mm inside the cuvette.

Procedure (specified distances depend on the size of the used pipette tips):

1. Press button ‘Initialize lift’.
2. Enter a distance (max. 60 mm). Start with e.g. 50 mm.
3. Press the downward directed arrow button
4. Fine tune distance with 1 mm steps

5. ‘Set new cuvette position’.
6. ‘Move lift to top’
7. Enter distance of +/- 38 mm and fine tune with 1 mm steps
8. ‘Set new middle position’
9. ‘Move lift to top’
10. ‘Close’.

See Figure 3.8 a and b, steps 1 to 10..

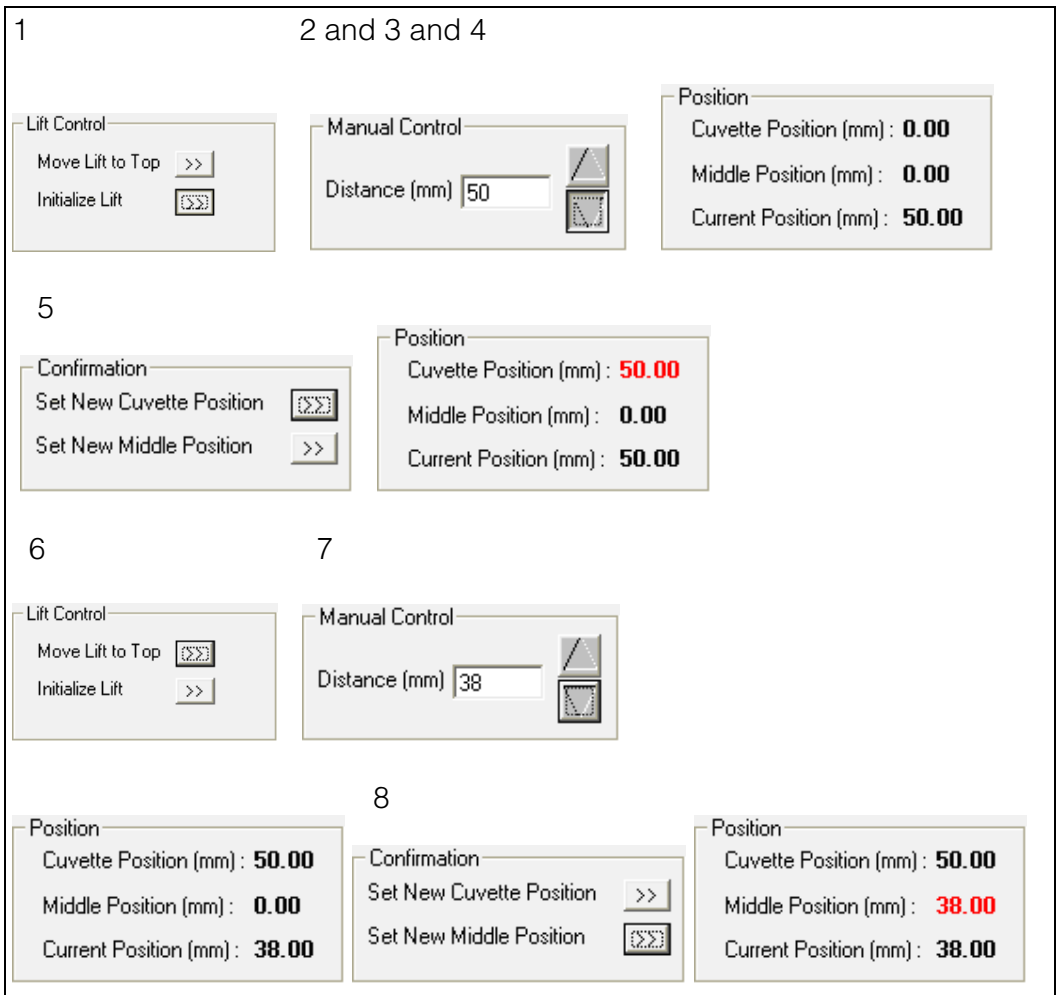


Figure 3.8 – a. The lift calibration procedure.

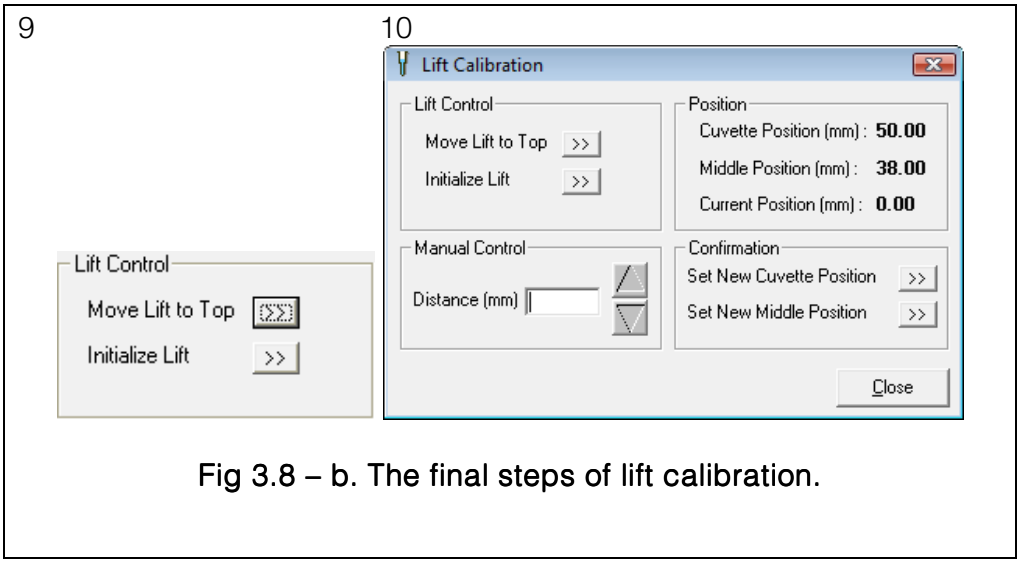


Fig 3.8 – b. The final steps of lift calibration.

3.4.6 – Installation of the gold sensor disk.

3.4.6.1 – Assembling the sensor disk on the hemi-cylinder.

Place a small drop of immersion oil on the outer edge of the hemi-cylinder and gently slide the modified gold disk (coated with 11-MUA) from the start to the end of the hemi-cylinder.

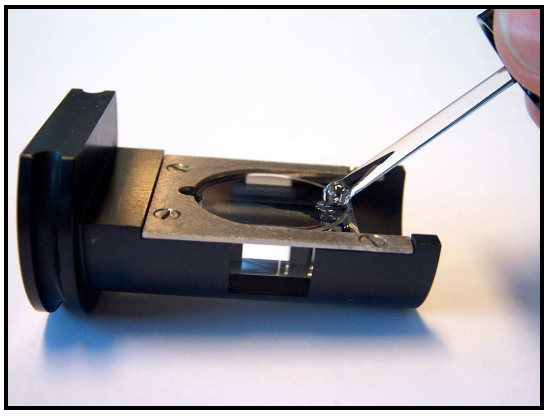


Figure 3.9 – A drop of immersion oil on top of the hemi-cylinder.

Be sure that the gold layer is facing up. A rim of gold can be seen at the gold coated side of the disk when slightly tilting it. Make sure that no air bubbles are introduced between the gold disk and the hemi-cylinder. Check this by looking through the hemi-cylinder.
Recommendations;

- Do not manipulate the gold sensor disk with bare hands. We recommend wearing gloves when handling the disk.

- Touch only the frosted sides of the hemi-cylinder to prevent scratches on the accurately polished round sides of the hemi cylinder.
- Manipulate the gold sensor disk with a fine tweezers, especially when you take it out of the plastic case.
- The gold sensor disk in the plastic case is oriented with the gold surface facing down.

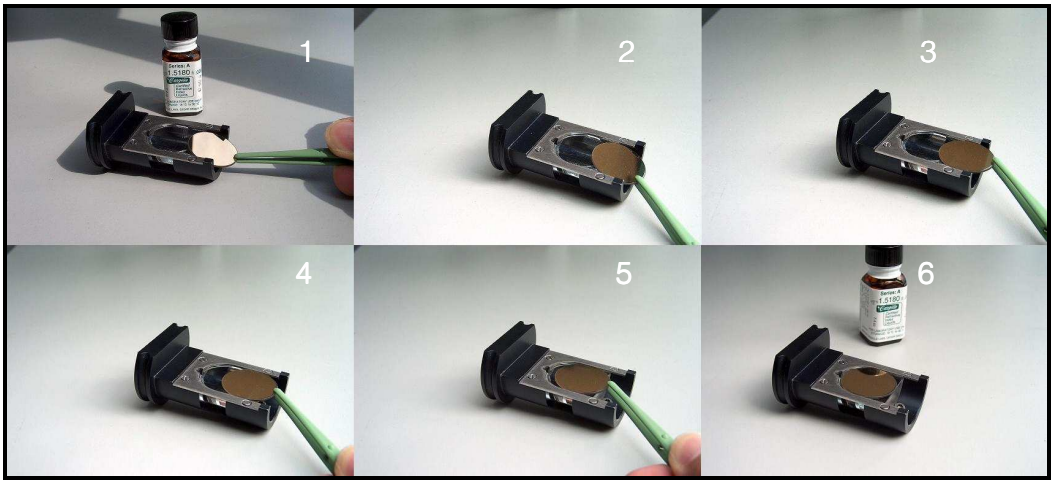


Figure 3.10 – Assembly of a disk.

Cleaning the hemi-cylinder and slider.

The slider and the hemi-cylinder need to be cleaned regularly because of inevitable spilling of immersion oil: Unscrew the M3x3 screw on the slider. Gently slide the hemi-cylinder out of the slider. Clean the hemi-cylinder with ethanol. Use only the lens tissue paper to clean and dry. Alternatively, the hemi-cylinder and slider can be cleaned in an ultra sonic water bath. After cleaning, slide the hemi-cylinder into the slider with the “oil overflow hole” to the oil overflow hole of the slider. Reassemble the M3 screw.

One Gold disk provides 5 to 7 measuring positions: Set a measuring position by gliding the disk over the hemi-cylinder surface with a clean pipette tip.

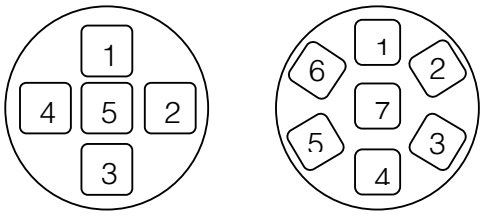


Figure 3.11 – Different positions on the gold disk.

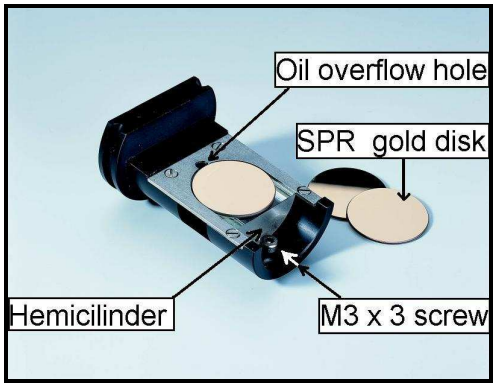
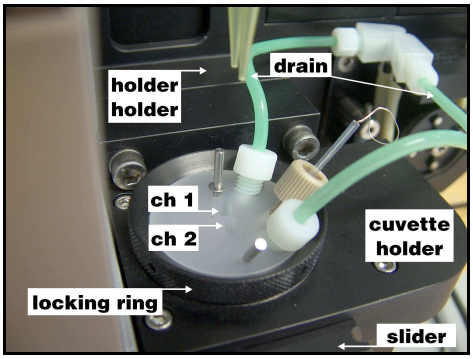


Figure 3.12 – Installed SPR gold disk.

3.4.6.2 – Installation of the cuvette.

There is only one way to position the cuvette in the cuvette holder. Position the cuvette with the pin towards the slot in the cuvette holder. The cuvette will slide in the cuvette-holder; tighten the cuvette with the ring. Make sure that the ring is tightened firmly to prevent leakage outside the channel. For extra tightening use the supplied SPR key.



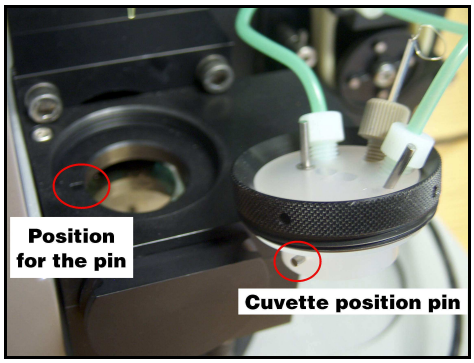


Figure 3.13 – LEFT- An overview of the cuvette holder. The SPRINGLE cuvette has only a channel ONE position)
Figure 3.14 – RIGHT- The 'positioning pin' of a cuvette.

3.4.7 – Check for leakage between the two measurement channels.

To check for leakage, pipette 125 µl HEPES into channel 1. Be sure the fluid reaches the gold disk. Check the SPR plot. To monitor the dip continuously, use the scope mode, which is available under the Options menu and on the toolbar. Deselect the scope mode by clicking the scope mode button again. If there is no leakage from one channel to the other, you will see a perfect dip

in channel 1 and a flat line around the absolute value of 90% in the empty channel 2 (total reflection). When in channel 2, the horizontal flat line goes down, the cuvette is not correctly assembled. Repeat the assembly of the cuvette until it is leakage free. Drain the HEPES from channel 1 and check channel 2 for leakage.

Awareness of possible leakage!

If there is leakage out of the cuvette onto the hemi-cylinder, the solution may come in contact with the detector. The measurements will become very noisy. A leakage with strong acids may detach the detector out of its calibrated position, causing a hardware problem.

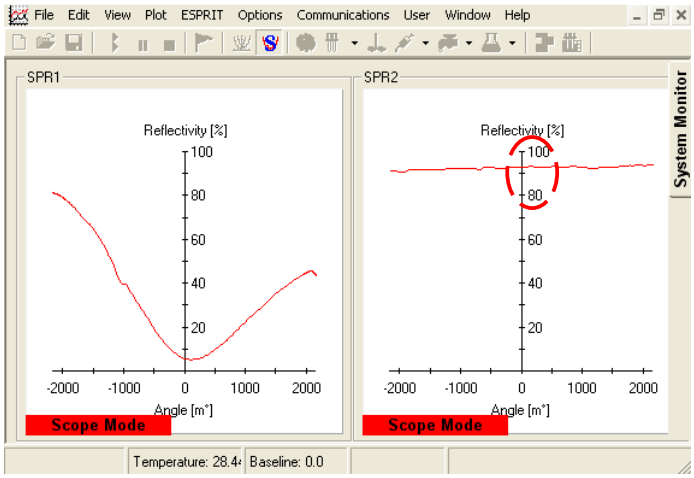


Figure 3.15 – Check for leakage from channel 1 into channel 2.

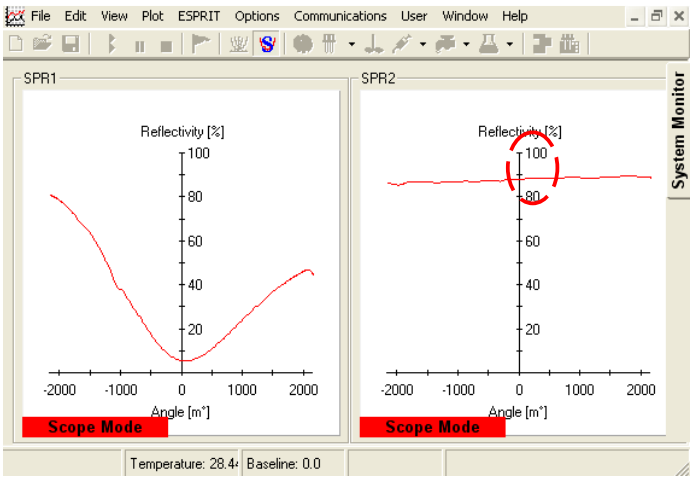


Figure 3.16 – Check for leakage from channel 1 into channel 2 .
Check for leakage from channel 1 to channel 2. Leakage! Compare with Fig. 3.15. After a longer period of time, a SPR minimum will appear.

In case of leakage, reassemble the sensor disk and check for leakage again.

3.4.8 – Fill tubing with buffer/Exchange the buffer solution.

Why should there be liquid in the tubing?
The solution in the tubing is used for washing the pipette tip. Secondly, fluid is not compressible like air, which results in highly accurate sample volumes and flow rates.

The general running buffer?
In particular applications with non specific interaction, buffer solutions and samples should contain 0.005% (v/v) Tween 20 to minimize non-specific adsorption to the sensor disk. Be aware of samples that are detergent-sensitive.

Chemicals: - Water, demineralised (demi), pro analysis, Merck 1.16754.9010
- HEPES free acid, MW 238.3, Fluka 54457

Preparation: -10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 0.005% Tween 20:
In 90 ml dissolve 238 mg HEPES free acid in 90 ml demiwater. Set pH to 7 with few drops of 10 M NaOH. Add 865 mg NaCl, 87.6 mg EDTA, 50 µl Tween 20 (10%). Adjust volume to 100 ml.

In a starting situation, the tubing can be empty (filled with air) or filled with a solution. Run the sequence “Initialization of instrument.seq” to fill the tubing with buffer or to change the running buffer as follows:
Select the menu bar item “options” - “Sequencer…”

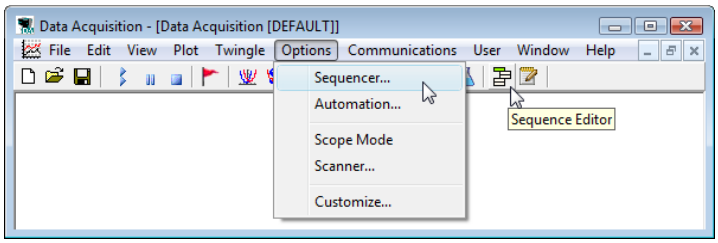


Figure 3.17 –Two ways to activate the Sequencer, via the Menu-Options or the Toolbar button.

1. Click “sequence” in the menu bar and select “Open sequence”.
2. Select from the list of sequences the file “Initialization of Instrument.SEQ”

3. Press the green arrow toolbar button to run the procedure to fill/exchange the tubing solution with the buffer from the buffer flask.
4. The procedure will flush the liquid handling system, which will give the opportunity to check for leaks at tubing connectors.

At this point the sensor disk can be exchanged. Do not forget to check for leakage after disk exchange.

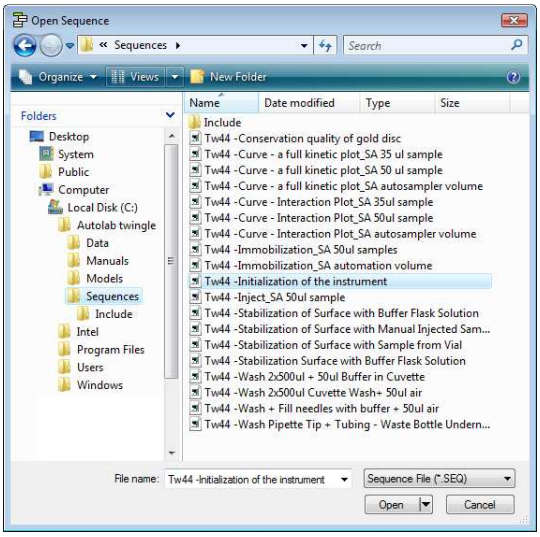


Figure 3.18 – TWINGLE; The sequence 'Initialization of Instrument.SEQ'.

The instrument is now ready for use; the 11-MUA modified gold disk and the cuvette are assembled correctly, the tubing is filled with the correct buffer.
The measurement can start. The experiments consist of two parts. The first part is to physically attach the Insulin protein molecule to the 11-MUA surface. This chemical binding step is called immobilization. The second part is the interaction of the sample anti-insulin antibody with the BSA protein molecule.

3.5 – D-day, The immobilization.

3.5.1 – Sample preparation.

For the immobilization procedure, prepare the following samples and solutions:

- Coupling buffer for baseline and wash steps;
  Preparation of *coupling buffer*;10 mM Acetate buffer pH 4.5:
  Dissolve 68,4 mg NaAc in 45 ml demi water. Adjust the pH to 4.5 with acetic acid. Adjust volume to 50 ml with demi water. The pH of the coupling buffer depends on the pI of the ligand protein. The general rule for the pH of coupling buffer is: $pH_{buffer} = pI_{ligand} - 0.5$

- EDC/NHS activation solution: 60 µl of 1:1 freshly mixed 0.4 M EDC and 0.1 M NHS in a 1.5ml vial;
  Preparation of 400 mM EDC solution:
  Dimethylaminopropyl-N'Ethylcarbodiimide N-3-hydrochloride, MW 191.70, Fluka 03449
  Weigh 153,4 mg of EDC in a 3 ml vial and dissolve it in 2 ml demi water.
  Preparation of 100mM NHS solution:
  N-Hydroxy Succinimide, MW 217.13, Fluka 56485
  Weigh 23 mg of NHS in a 3 ml vial and dissolve it in 2 ml demi water.

- Ligand sample: 200 µl of ligand solution dissolved in coupling buffer in a 1.5 ml vial;
  Preparation of 5 µg/ml insulin to immobilize on the sensor disk;
  Insulin from bovine pancreas, Mw 5733.49, Sigma I5500.
  Dissolve 1 mg insulin in 1 ml 1 M Acetic acid. Dilute 50 µl 200 x with 950 µl 10 mM Acetate buffer

- Deactivation solution: 200 µl of 1 M ethanolamine pH 8.5 in a 1.5 ml vial;
  Preparation of 1 M Ethanolamine solution:
  Pipette 600 µl of Ethanolamine in a 25 ml flask, dilute it with 10 ml demi water, and adjust the pH to 8.5 with 1 M HCl.

- Regeneration solution: Coupling buffer + 0.1% SDS
  SDS; Sodium Dodecyl Sulphate or Lauryl sulphate sodium salt, 20% in H_2O, MW 288.38, Fluka 05030

When to prepare the solutions?

- EDC and NHS are not stable in solution. Once prepared, use it the same day and/or store aliquots of 200 µl at -20 C°.
- The pH of the acetate buffer can change in time, so check the pH before use.
- The ligand dissolved in acetate buffer should be freshly prepared. The same preparation of acetate buffer should be used for all steps of the immobilization measurement.
- Diluted antibodies can be stored at 4 C° for a few days, but be careful! The experiment baseline buffer should be the same as the buffer for the dilution of the samples.

3.5.2 – Set angle position of the sodium acetate buffer.

Put 50 µl of acetate buffer on the gold disk and check the dip by selecting the Scope Mode button . To deactivate the scope mode, press this button again. The scope mode will update the SPR angle every 0.5 seconds.

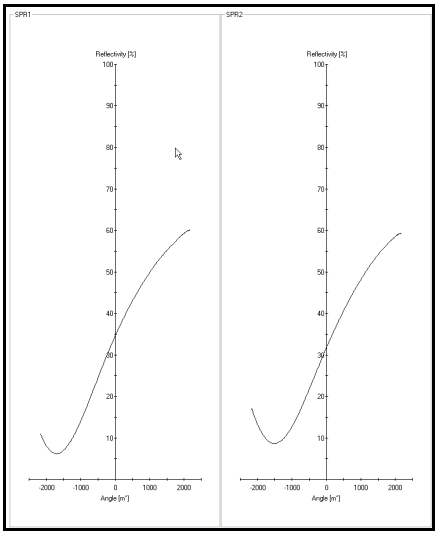


Figure 3.19 – SPR "dip".

Figure 3.20 – The optical path cover.

Every TWINGLE instrument is calibrated with water on a bare gold disk. The lowest value of the SPR 'dip' is between 0 and 10 percent absolute reflection.

To change the position of the dip, release the retaining screw of the optical path. Adjusting the position of the dip is done by turning the micrometer spindle. Click on start measurement in the tool bar and follow the change of the angle in real time. Set the baseline around -1500 millidegrees (m°). After adjusting the baseline, fasten the retaining screw again.

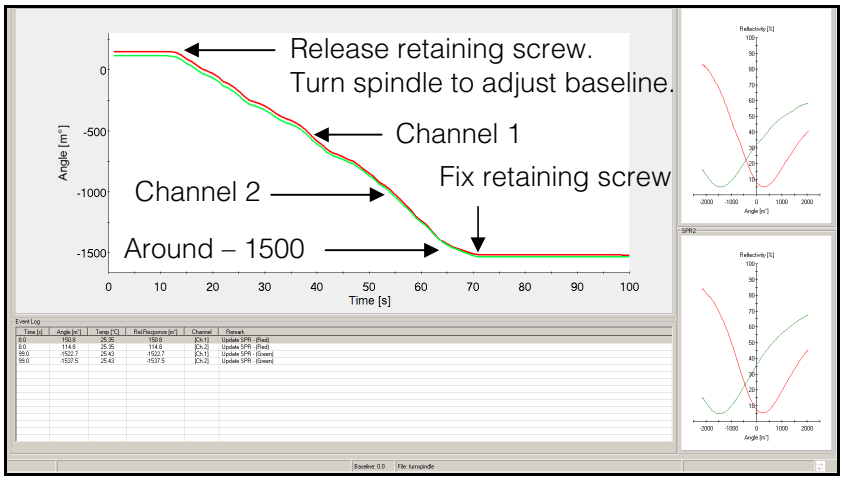


Figure 3.21 – Adjustment of the baseline angle before immobilization.

Why should the sodium acetate buffer SPR angle be set at -1500 mº?

The solutions used in the immobilization differ significantly in refractive index and will change a number of times during the procedure. The acetate buffer has the lowest refractive index and therefore the smallest SPR angle; the Ethanolamine solution has the largest angle.

3.5.3 – Stabilize / rehydrate the dry 11-MUA disk.

Before the modified gold disk can be used for immobilization, the baseline must be stabilized.

The Coupling Buffer will be used for all washing steps in the immobilization experiment. Therefore, the buffer flask will be filled with this solution.

Stabilize the surface of the gold disk using one of the sequences:

- Stabilization with buffer from flask.SEQ
- Stabilization with manually injected sample.SEQ
- Stabilization with sample from vial.SEQ

Start the sequence and continue to wash until the baseline is sufficiently stable.

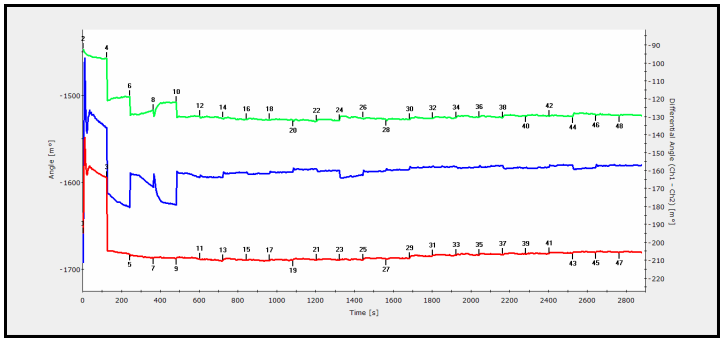


Figure 3.22 – Stabilization/cleaning of the gold disk surface with coupling buffer (B4).

Eventually, every solution should show the same SPR angle every time it is dispensed on the surface. When the desired stability is reached, the sequence can be stopped at any time by clicking the stop measurement button in the tool bar.

Stabilization of the surface is necessary for all kinds of modified gold surfaces!

Commercially available Dextran surfaces also need extensive cleaning. In general, change the baseline solution one after the other, like 0.1M NaOH and 0.1M HCl alternatively. Eventually, every solution should show the same SPR angle every time it is dispensed on the surface.

3.5.4 – Start the immobilization procedure.

The EDC/NHS immobilization procedure is a standardized procedure that can be easily performed semi-automatically. Open the sequence editor window, and select the file "Tw44 –immobilization_SA 50ul samples.SEQ" to have a glance on the procedure.

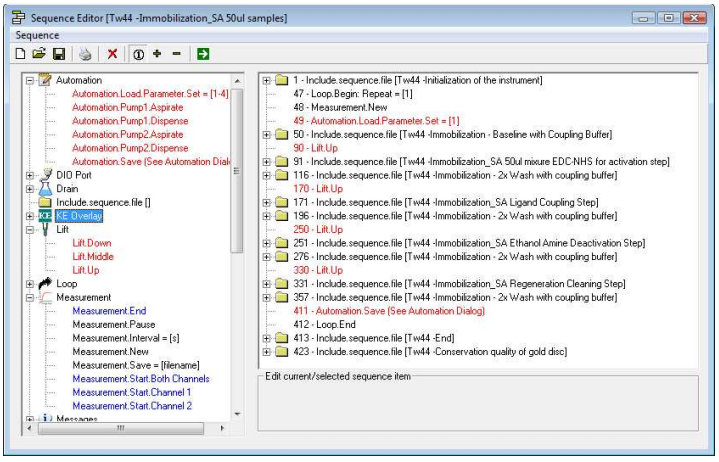

Sequence Editor [Tw44 -Immobilization_SA 50ul samples]
Sequence
Automation
Automation.Load.Parameter.Set = [1-4]
Automation.Pump1.Aspirate
Automation.Pump1.Dispense
Automation.Pump2.Aspirate
Automation.Pump2.Dispense
Automation.Save (See Automation Dial
DIO Port
Drain
Include.sequence.file []
KE Overlay
Lift
Lift.Down
Lift.Middle
Lift.Up
Loop
Measurement
Measurement.End
Measurement.Pause
Measurement.Interval = [s]
Measurement.New
Measurement.Save = [filename]
Measurement.Start.Both Channels
Measurement.Start.Channel 1
Measurement.Start.Channel 2
1 - Include.sequence.file [Tw44 -Initialization of the instrument]
47 - Loop.Begin: Repeat = [1]
48 - Measurement.New
49 - Automation.Load.Parameter.Set = [1]
50 - Include.sequence.file [Tw44 -Immobilization - Baseline with Coupling Buffer]
90 - Lift.Up
91 - Include.sequence.file [Tw44 -Immobilization_SA 50ul mixure EDC-NHS for activation step]
116 - Include.sequence.file [Tw44 -Immobilization - 2x Wash with coupling buffer]
170 - Lift.Up
171 - Include.sequence.file [Tw44 -Immobilization_SA Ligand Coupling Step]
196 - Include.sequence.file [Tw44 -Immobilization - 2x Wash with coupling buffer]
250 - Lift.Up
251 - Include.sequence.file [Tw44 -Immobilization_SA Ethanol Amine Deactivation Step]
276 - Include.sequence.file [Tw44 -Immobilization - 2x Wash with coupling buffer]
330 - Lift.Up
331 - Include.sequence.file [Tw44 -Immobilization_SA Regeneration Cleaning Step]
357 - Include.sequence.file [Tw44 -Immobilization - 2x Wash with coupling buffer]
411 - Automation.Save (See Automation Dialog)
412 - Loop.End
413 - Include.sequence.file [Tw44 -End]
423 - Include.sequence.file [Tw44 -Conservation quality of gold disc]
Edit current/selected sequence item

Figure 3.23 – The sequence editor showing the sequence "Tw44 – Immobilization_SA 50ul samples.SEQ".

The experiment sequence set up;

1. baseline,
   Read include sequence "Tw44 –Immobilization – Baseline with Coupling buffer.seq" (starts at command line 50)
2. EDC/NHS activation,
   Read include sequence "Tw44 – Immobilization_SA 50ul mixure EDC-NHS for activation step.seq" (starts at command line 91)
3. Ligand coupling,
   Read include sequence "Tw44 – Immobilization_SA Ligand coupling Step.seq" (starts at command line 171)
4. Ethanol Amine Deactivation

Read include sequence “Tw44 – Immobilization_SA Ethanol Amine Deactivation step.seq” (starts at command line 251)

5. Regeneration/cleaning
   Read include sequence “Tw44 – Immobilization_SA Regeneration cleaning step.seq” (starts at command line 331)

Within those include sequences, commands for incubation times are written, subsequently, (1) Wait.Baseline [s] (line 86), (2) Wait.Associate [s] (line 113), (3) Wait.Interval.2 [s] (line 193), (4) Wait.Interval.3 [s] (line 273), (5) Wait.Regenerate [s] (line 354)
Those times are linked with the number filled out in the automation window.

- Define the analysis time settings:

Baseline	120s	= coupling buffer
EDC/NHS activation	300s	= EDC/NHS activation time
Ligand coupling	900s	= ligand coupling time
Deactivation	600s	= deactivation time
Regeneration	120s	= regeneration time

The automation window enables to perform the immobilization semi-automatically.

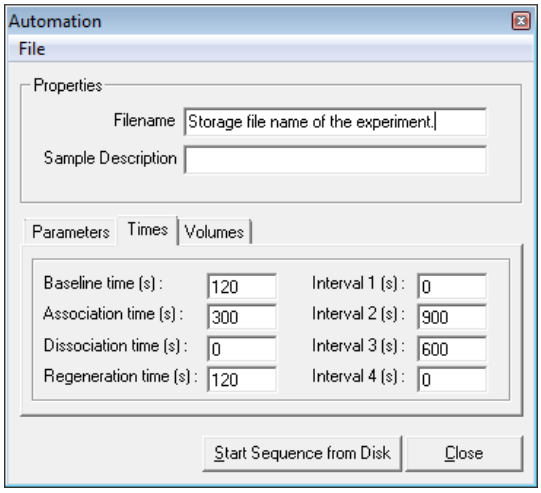


Figure 3.24 – The automation window with three tab sheets to set up the experiment.

When the desired interval times are set (see fig.3.24), the TAB sheet “Parameters” can be used to adjust system parameters for the experiment.

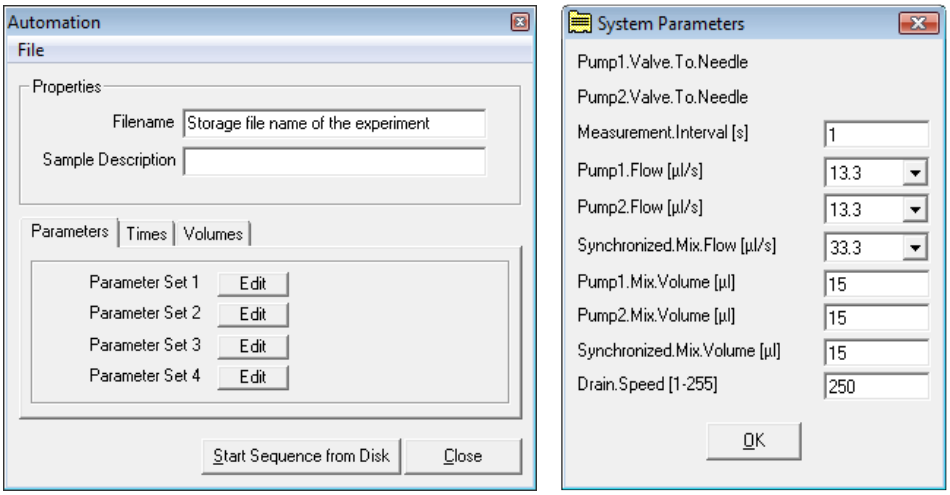


Figure 3.25 – The automation window with Parameters tab sheets to set up the experiment using EDIT.

With the button EDIT a new window "System parameters" pops up. Within this window every item can be adjusted. The settings will be used for the experiment and loaded into the sequence with the command line 49 "Automation.Load.Parameters.Set = [1]"

To finish the Automation window;

- Give the experiment a name under which it will be stored.
  filename (example) immobilization,
  Because of automatic incremental numbering, the file names will start with immob001 and every next experiment with the same name will be up-numbered up to immob999.

- Select the 'Start sequence from disk' button at the bottom of the automation control window (Figure 3.25) to select and to execute the specific immobilization experiment.

Have the samples ready for the experiment.;
Sample Injection routine;
Vial 1/ sample 1: 75 µl EDC
Vial 2/ sample 2: 75 µl NHS

When the EDC/NHS sample is requested by the software, mix both chemical components and present it to the pipette tip for incubation. The chemicals are not alouwed to be mixed sooner.

Vial 3/sample 3: Pipette tip 1: 75 µl ligand Insulin in acetate buffer
Vial 4/sample 4: Pipette tip 2: 75 µl acetate buffer
Vial 5/sample 5: 200 µl 1M ethanolamine pH 8.5
Vial 6/sample 6: 500 µl regeneration buffer; 0.1 M HCl

The Coupling Buffer is being used for all washing steps in the immobilization experiment. Therefore, the buffer flask will be filled with this solution.

3.6 – The interaction.

3.6.1 – Sample preparation.

For the interaction procedure, prepare the following samples and solutions:

Chemicals;
HEPES free acid, MW 238.3, Fluka 54457
EDTA; Ethylenediaminetetraacetic acid solution, MW 292.24, Fluka 03690, pH 8.0, 0.5 M in H2O,
NaCl, 58.44 g/Mol, Sigma-Aldrich S7653
Tween 20, 10% in water, Fluka 93774
Sodium dodecyl sulfate (SDS) 10% solution Sigma 71736

Preparation of *running buffer*;
- 10mM HEPES, 150 mM NaCl, 3 mM EDTA, 0.005% Tween 20:
  In 90 ml dissolve 238 mg HEPES free acid in 90 ml demiwater. Set pH to 7 with few drops of 10 M NaOH. Add 865 mg NaCl, 87.6 mg EDTA, 50 µl Tween 20 (10%). Adjust volume to 100 ml.

Preparation of Regeneration solution;
- 10 mM Acetate buffer + 0.1%SDS

Preparation of anti-Insulin dilutions:

S stock (1:500):
Pipette 2 µl of the concentrated stock solution anti-insulin in a vial of 1.5 ml, dilute this with 1000 µl HEPES buffer. Mix by pipetting several times up and down. This is a 1:500 dilution

1 Dilution 1 (1:8000):
Pipette 60 µl from the stock solution into a 1.5 ml vial and add 900 µl HEPES buffer.

2 Dilution 2 (1:16000):
Pipette 200 µl from dilution 1 into a 1.5 ml vial and add 200 µl HEPES buffer.

3 Dilution 3 (1:32000):
Pipette 200 µl from dilution 2 into a 1.5 ml vial and add 200 µl HEPES buffer.

3.6.2 – Stabilize the surface.

Change the coupling buffer in the flask with the HEPES buffer (use the "initialization of instrument.seq"). Stabilize the surface as described in section 3.5.3 of the immobilization paragraph. Thiol layers, Dextran layers or surfaces with immobilized ligands have to be stabilized to minimize matrix effects that are caused by differences in pH or ionic strength (high-low salt concentrations) of the different buffers used throughout the experiment. The matrix effects influence the SPR signal. Due to exposure of the layer with the different buffers of the experiment, the layer will respond in a more predictive way and will continuously give a SPR signal at the same angle. When the desired stability is reached, the sequence can be stopped at any time by clicking the "Stop measurement" button in the tool bar.

3.6.3 – Start the association procedure.

The association procedure starts with using the Automation window. First get some inside knowledge of the procedure itself. Open the Sequence editor and browse for the "Tw44 –Curve – a full kinetic plot_SA 50ul sample.SEQ"

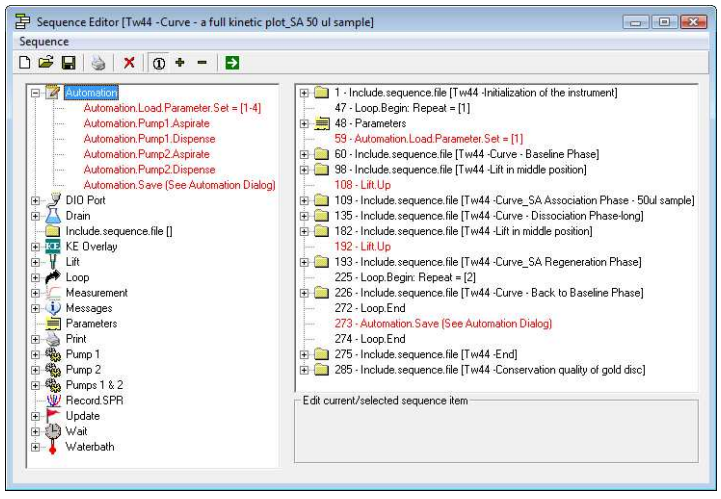


Figure 3.26 – The sequence editor showing the sequence " Tw44 – Curve – a full kinetic plot_SA 50ul sample.SEQ".

The experiment sequence set up;

1. Baseline,
   Read include sequence "Tw44 -Curve - Baseline Phase.seq" (starts at command line 60)
2. Association,

Read include sequence “Tw44 -Curve_SA Association Phase - 50ul sample.seq” (starts at command line 109)

3. Dissociation,
   Read include sequence “Tw44 -Curve - Dissociation Phase-long.seq” (starts at command line 135)
4. Regeneration,
   Read include sequence “Tw44 -Curve_SA Regeneration Phase.seq” (starts at command line 192)
5. Back to Baseline,
   Read include sequence “Tw44 -Curve - Back to Baseline Phase.seq” (starts at command line 226)

Within those include sequences, commands for incubation times are written, subsequently, (1) Wait.Baseline [s] (line 94), (2) Wait.Associate [s] (line 132), (3) Wait.Dissociate [s] (line 179), (4) Wait.Regenerate [s] (line 222), (5) Wait.Interval 1 [s] (line 269).
Those times are linked with the number filled out in the automation window.

- Define the analysis time settings:

Baseline	120s	= baseline time
Association	600s	= association time
Dissociation	60s	= dissociation time
Regeneration	120s	= regeneration time
Back to baseline	120s	= baseline time

The automation window enables to perform the binding experiment semi-automatic.

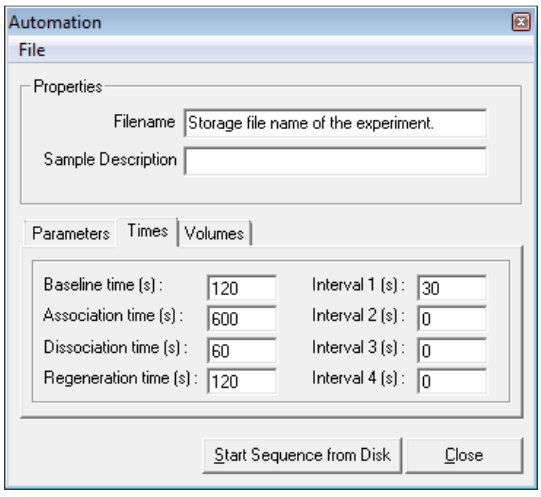


Figure 3.27 – The automation window with three tab sheets to set up the experiment.

When the desired interval times are set (see fig.3.27), the TAB sheet “Parameters” can be used to adjust system parameters for the experiment.

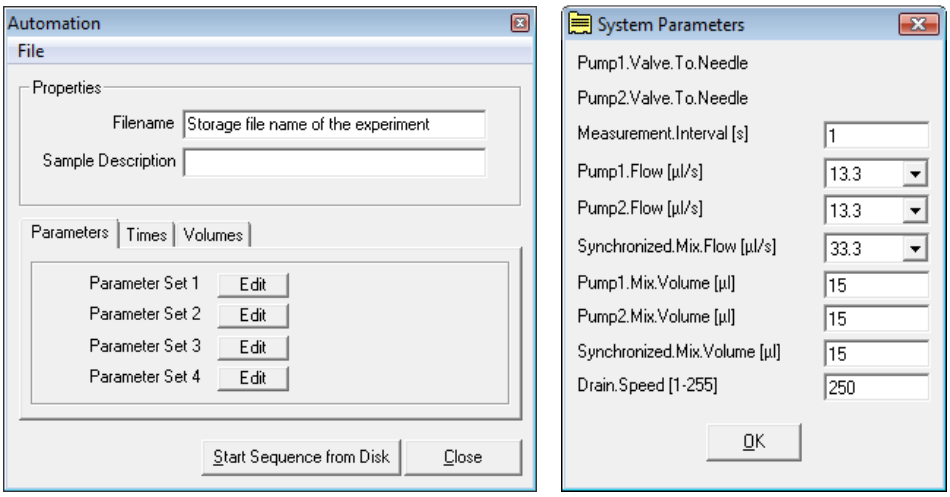


Figure 3.28 – The automation window with Parameters tab sheets to set up the experiment using EDIT.

With the button EDIT a new window "System parameters" pops up. Within this window every item can be adjusted. The settings will be used for the experiment and loaded into the sequence with the command line 59 "Automation.Load.Parameters.Set = [1]"

To finish the Automation window;

- Give the experiment a name under which it will be stored.
  filename (example) interaction,
  Because of automatic incremental numbering, the file names will start with interact001 and every next experiment with the same name will be up-numbered up to interact999.

- Select the 'Start sequence from disk' button at the bottom of the automation control window (Figure 3.27) to select and to execute the specific experiment.

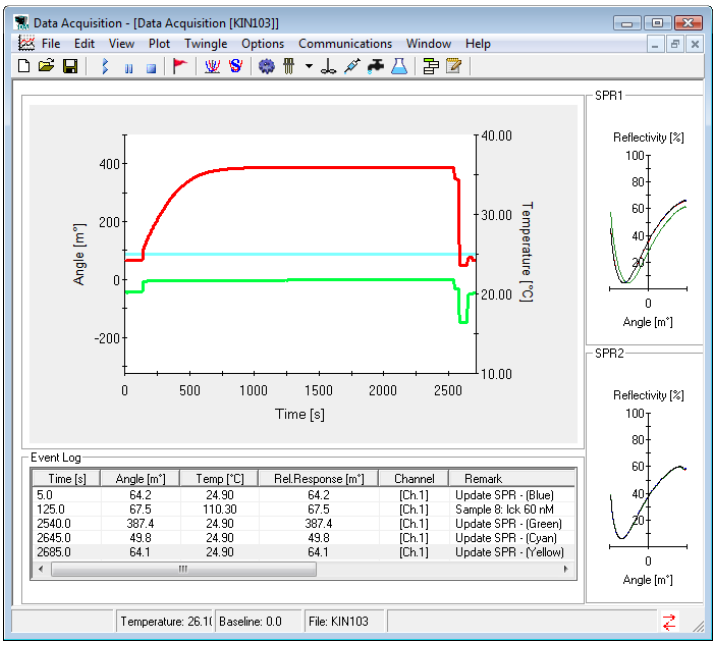
Have the samples ready for the experiment.;
Sample Injection routine;

Vial 1/sample 1: Pipette tip 1: 75 µl analyte anti-Insulin (dilution1) in HEPES buffer
Vial 2/sample 2: Pipette tip 2: 75 µl HEPES buffer
Vial 3/sample 3: Pipette 1 and 2: 1000 µl Regeneration solution

The HEPES Buffer is being used for all washing steps in the interaction experiment. Therefore, the buffer flask will be filled with this solution.

Next experiments can be performed with the other prepared dilutions 2 and 3.

3.7 – The Autolab Twingle data.


Data Acquisition - [Data Acquisition [KIN103]]
File Edit View Plot Twingle Options Communications Window Help
Angle [m°]
Temperature [°C]
Time [s]
SPR1
Reflectivity [%]
Angle [m°]
SPR2
Reflectivity [%]
Angle [m°]
Event Log
Time [s] Angle [m°] Temp [°C] Rel.Response [m°] Channel Remark
5.0 64.2 24.90 64.2 [Ch.1] Update SPR - (Blue)
125.0 67.5 110.30 67.5 [Ch.1] Sample 8: Ick 60 nM
2540.0 387.4 24.90 387.4 [Ch.1] Update SPR - (Green)
2645.0 49.8 24.90 49.8 [Ch.1] Update SPR - (Cyan)
2685.0 64.1 24.90 64.1 [Ch.1] Update SPR - (Yellow)
Temperature: 26.1(
Baseline: 0.0
File: KIN103

3.8 – Cleaning of the TWINGLE instrument.

This is a guiding principle for cleaning all parts in the system which are in contact with the solutions used in the experiments. Replace the buffer flask solution step by step with cleaning solution after the specific sequence is finished.

Cleaning Solution 1: - 0.5% (w/v) SDS/ 1% (w/v) Triton in water
Total cleaning time about 10 min.

Cleaning Solution 2: - 0.5% (w/v) SDS
Total cleaning time about 10 min.

Cleaning Solution 3: - 50 mM Glycine-NaOH pH 9.5
Total cleaning time about 10 min.

Cleaning solution 4: - 6 M Urea
Total cleaning time about 10 min.

Cleaning solution 5: - 1% acetic acid
Total cleaning time about 20 min.

Cleaning solution 6: - 0.2 M NaHCO3
Total cleaning time about 10 min.

Cleaning solution 7: - Hydrochloric acid : 0.1 M HCl
Total cleaning time about 20 min.

Cleaning solution 8: - Water
Total cleaning time about 10 min.

Cleaning solution 9: - 70% Ethanol
Total cleaning time about 10 min.

A. Clean before shutting down for a weekend;
    - Use the sequence "*; 'Initialization of Instrument.SEQ'*
    - Put the inlet buffer flask tubings out off the flask
    - Run the sequence to empty all tubings
    - It's also OK to replace the solution in the tubings with Solution 7, water
B. Clean needles, cuvette and connected tubings once every two weeks
    - Use the sequence "*; 'Initialization of Instrument.SEQ'*
    - Place all inlet tubings into the 'buffer' flask
    - Run the sequence to clean with solution 1 and 8
C. Total clean of the system every two month's
    - Use the sequence "*; 'Initialization of Instrument.SEQ'*
    - Place all inlet tubing into the 'buffer' flask
    - Run the sequence using every solution 1, 3, 7, 8, 9 step by step
D. Total clean of the system every half year
    - Use the sequence "*; 'Initialization of Instrument.SEQ'*
    - Place all inlet tubing into the 'buffer' flask
    - Run the sequence using every solution 2, 4, 5, 6, 8, step by step

Use the routine *'Initialization of Instrument.SEQ'* to prepare the system before use.
Running buffer 1: PBS pH 7.4
Or Running buffer 2: 10 mM HEPES, 150 mM NaCl, 3.4 mM EDTA, 0.005% Tween P20

Chapter 4

4 – Getting started Autolab SPRINGLE.

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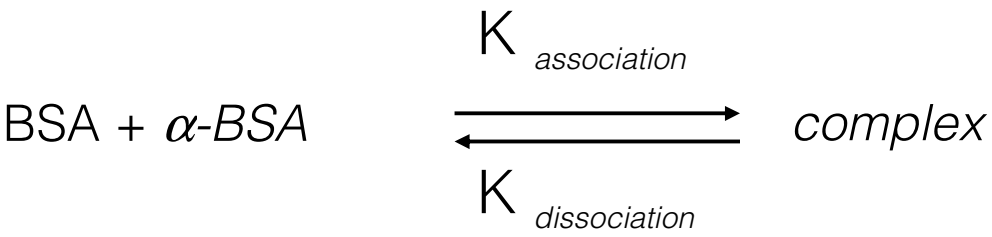
4.2 – Introduction.

If this chapter is unclear please read chapter 3, which has a different setup with the same content.

This "getting started" document will take you step by step through the initialization of the TWINGLE/SPRINGLE instrument and, subsequently, through an interaction experiment where the antibody anti-BSA will be interacting with the immobilized protein BSA. Throughout this procedure, most features of the software will be illustrated.

It is presumed that the hardware and software have been installed before; the cuvette and hemi-cylinder are disassembled and all tubing is empty. A 60 min. warm-up time of the Autolab SPRINGLE should be taken into account.

Detection of binding events between Insulin and anti-Insulin



First, the modified gold layer on the sensor disk is coated with mercapto-undecanoic acid (11-MUA). After being assembled into the instrument, the modified surface is stabilized with the immobilization coupling buffer. In the immobilization procedure (Immobilization), the acid group of this molecule is activated by incubation with EDC and NHS. Subsequently, the Insulin is immobilized onto the modified gold layer.
The Insulin layer will thereafter be stabilized with association buffer. In the interaction phase (Interaction), several dilutions of anti-Insulin are used to visualize the BSA / anti-BSA interaction. In the reference channel the effect of the plain association buffer is recorded to correct for any a-specific interaction factors. After the association phase, which is used to calculate the association constant Ka, the dissociation phase is performed by washing the sample away with association buffer. The association buffer is the same buffer as the dilution buffer for the anti-BSA. The dissociation phase is used for determining the strength of the interaction. After the dissociation phase, all bound anti-Insulin is removed from the Insulin coated gold disk by diluted SDS, Sodium dodecyl sulphate, (regeneration buffer). Then, the baseline of the modified disk will be restored by washing the regeneration solution replacing it with the baseline HEPES buffer.

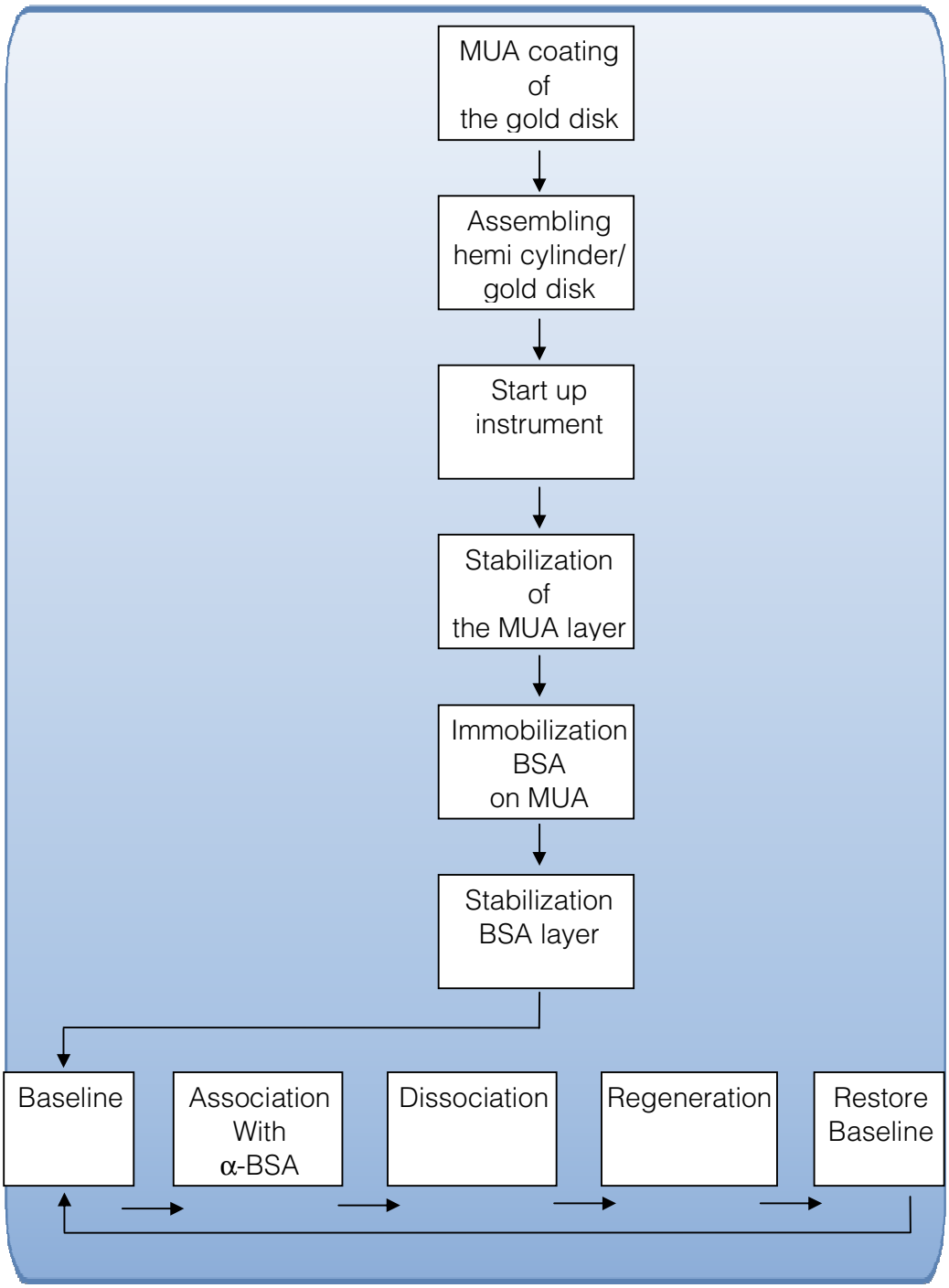


Figure 4.1 – Flow chart of the experimental setup.

After one initial immobilization, numerous SPR experiments can be performed. The surface plasmon resonance (SPR) measures angle versus time. There is a linear relationship between the amount of bound material and shift in SPR angle. The SPR angle shift, in millidegrees (m°), is used as a response unit to quantify the binding of macromolecules to the sensor surface. A change of 122 m° represents a change in surface protein mass of approximately 1 ng/mm^2.

4.3 – Startup of the Autolab SPRINGLE instrument.

- Install the power supply cable and connect the com port of the instrument to the computer with the RS232 connector cable.
- Switch on the Autolab SPRINGLE instrument.
- Use the main switch on the back panel (Figure 4.2) and the power button situated at the right top position of the front panel. The LED in the power button lights up after a few seconds.
- Start the Autolab SPR Data Acquisition software. Wait until the instrument has finished initiating the syringe pump

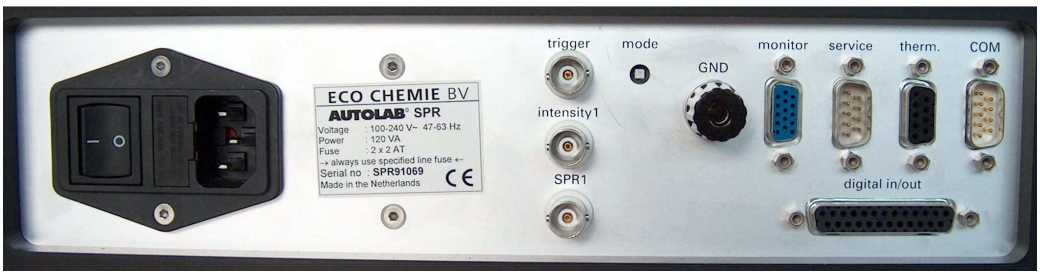


Figure 4.2 – the back panel of the Autolab SPRINGLE.
The black power switch is situated on the left.

4.4 – Preparation of solutions.

What do I use as a general running buffer?

A 10mM Phosphate buffered saline (PBS) solution is recommended as a general wash/running buffer. In this particular application with proteins, buffer solutions and samples should contain 0.005% (v/v) Tween 20 to minimize non-specific adsorption to the sensor disk. Be aware of samples that are detergent-sensitive.

4.4.1 – Chemicals.

A.1 11 – Mercapto-undecanoic acid (11-MUA), Aldrich 450561
A.2 10x PBS buffer (Phosphate Buffered Saline, 0.1M, 9% NaCl), Fluka 79383
A.3 Water, demineralised (demi), pro analysis, Merck 1.16754.9010
A.4 Hydrochloric Acid (HCl), 30% (= 9.46M) Fluka 17077
A.5 BSA, Albumin from Bovine Serum, Fluka 05477
A.6 Anti-BSA, Rabbit anti-Cow Albumin, DAKO Z0229; or Clone BSA-33 Sigma B2901

A.7 Alcohol, pro analysis 99,5 %; propanol or ethanol or methanol
A.8 NHS, N-Hydroxy Succinimide, Fluka 56480
A.9 EDC/ EDC-HCl. Dimethylaminopropyl-N'Ethylcarbodiimide N-3-hydrochloride, Fluka 03449
A.10 Ethanolamine, Fluka 02400
A.11 Sodium Acetate – trihydrate ($NaAc.3H_2O$); Fluka 71190.
A.12 Acetic Acid, Sigma A6283
A.13 Tween 20, 10% in water, Fluka 93774
A.14 HEPES free acid, Fluka 54457, MW 238.3 g/Mol
A.15 EDTA (ethylenediamine tetraacetic acid), MW 292.25 g/Mol
A.16 NaCl 58.44 g/Mol

4.4.2 – Reagents.

B.1 Preparation of 1 mM 11-Mercaptoundecanoic acid (11-MUA):
dissolve 11 mg 11-Mercaptoundecanoic acid (Mw. 218.36) in 50ml alcohol; like ethanol, ethanol or propanol (A.7).

B.2 Preparation of PBS buffer, 10mM:
Dilute PBS buffer in demineralised (demi) water. Pipette 20 ml of concentrated PBS (A.2) in a 300 ml flask and add 180 ml of demiwater. It is recommended to filter the solution through a 0.22 µm filter. Degass under vacuum.

B.3 Preparation of *regeneration buffer*; 0.1M and 1 M hydrochloric acid (HCl, A.4):
Dilute 0.5 ml of the 30% (9.46M) HCl in 47 ml demi water to get 0.1M HCl.
Dilute 5 ml of the 30% (9.46M) HCl in 42,5 ml demi water to get 1.0 M HCl.

B.4 Preparation of *coupling buffer*;10 mM Acetate buffer (A.11) pH 4.5:
Dissolve 68,4 mg NaAc (Mw. 136.08) in 45 ml demi water. Adjust the pH to 4.5 with acetic acid (A.12). Adjust volume to 50 ml with demi water. The pH of the coupling buffer depends on the pI of the ligand protein. The general rule for the pH of coupling buffer is: $pH_{buffer} = pI_{ligand} - 0.5$

B.4a Preparation of *association buffer*; 10mM HEPES, 150mM NaCl, 3mM EDTA, 0.005% tween 20:
In 90 ml dissolve 238mg HEPES free acid in 90 ml demiwater. Set pH to 7 with few drops of 10M NaOH. Add 865 mg NaCl, 87.6 mg EDTA, 50 µl Tween 20(10%). Adjust volume to 100 ml.

B.5 Preparation of 1mg/ml BSA to immobilize on the sensor disk:
Dissolve10 mg BSA (A.5) in 1 ml 10mM Acetate buffer (solution B.4). Dilute 100 µl 10x with 900 µl 10mM Acetate buffer (B.4)

B.6 Preparation of 100mM NHS solution:
Weigh 23 mg of NHS (Mw 115.09, A.8) in a 3 ml vial and dissolve it in 2 ml demi water.

B.7 Preparation of 400mM EDC solution:

Weigh 153,4 mg of EDC (Mw. 191.70; A.9) in a 3 ml vial and dissolve it in 2 ml demi water.

B.8 Preparation of 1M Ethanolamine solution:
Pipette 600 µl of Ethanolamine in a 25 ml flask, dilute it with 10 ml demi water, and adjust the pH to 8.5 with 1 M HCl.

Preparation of anti-BSA dilutions:

B.9 Dilution 1 (1:100):
Pipette 5µl of the concentrated stock solution anti-BSA (A.6) in a vial of 1.5 ml, dilute this with 500 µl HEPES buffer. Mix by pipetting several times up and down.

B.10 Dilution 2 (1:300):
Pipette 100 µl from dilution 1 into a 1.5 ml vial and add 200µl HEPES buffer.

B.11 Dilution 3 (1:900):
Pipette 100 µl from dilution 2 into a 1.5 ml vial and add 200µl HEPES buffer.

When to prepare the solutions?

- EDC and NHS are not stable in solution. Once prepared, use it the same day and/or store aliquots of 200 µl at -20 C°.
- The pH of the acetate buffer can change in time, so check the pH before use.
- The ligand dissolved in acetate buffer should be freshly prepared. The same preparation of acetate buffer should be used for all steps of the immobilization measurement.
- Diluted antibodies can be stored at 4 C° for a few days

4.5 – Liquid Handling set up Autolab SPRINGLE.

Before the system can be used, all tubing needs to be filled with buffer. Fill buffer flask with PBS buffer and insert the inlet tubing of the syringe pump into the running buffer flask and the green outlet tubing (in figure 4.3 shown in red) of the peristaltic ‘drain’ pump into the waste bottle.

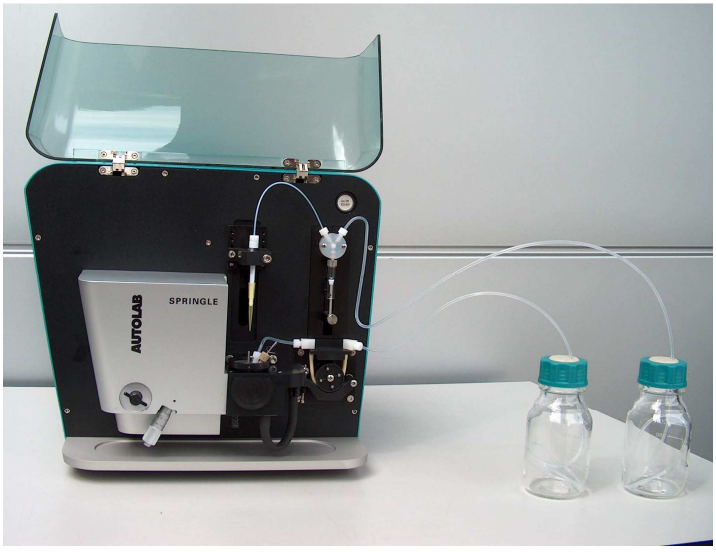


Figure 4.3 – The draining tube from the drain peristaltic pump is inserted into the waste bottle (green tubing in reality). The syringe tube is inserted into the buffer flask.

4.6 – Initialization of the SPRINGLE instrument.

Before using the instrument:

- Calibrate lift positions.
- Prepare the gold disk
- Assemble a new sensor disk
- Assemble the cuvette

- new sensor disk
- Assemble the cuvette

4.6.1 – Autolab SPRINGLE lift position calibration.

There are three ways to find out if the lift is calibrated;

- In the menu SPRINGLE find the 'lift position' item to check the positions, up, middle, down,
- In the menu SPRINGLE find the 'Manual control' item to check the three positions,
- In the toolbar find the button 'lift positions'

Open the 'Manual Control' window in the 'SPRINGLE' Menu or with the button in the toolbar

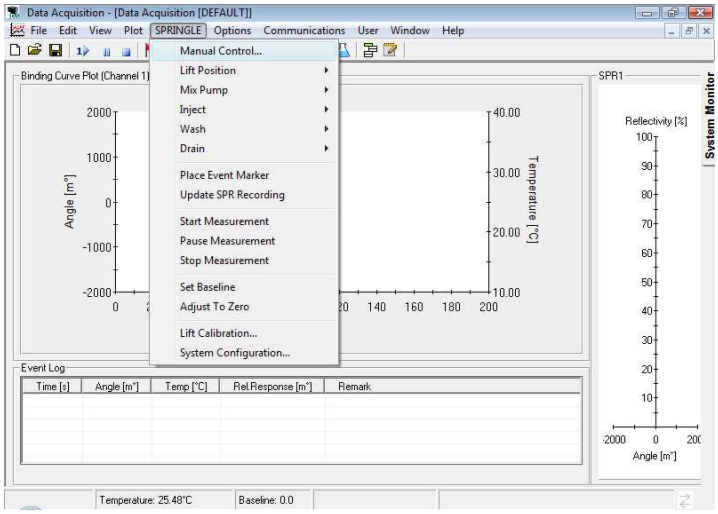


Figure 4.4 – Menu SPRINGLE to open 'Manual Control' window.

The lift position has to be calibrated every time you change the pipette tip.

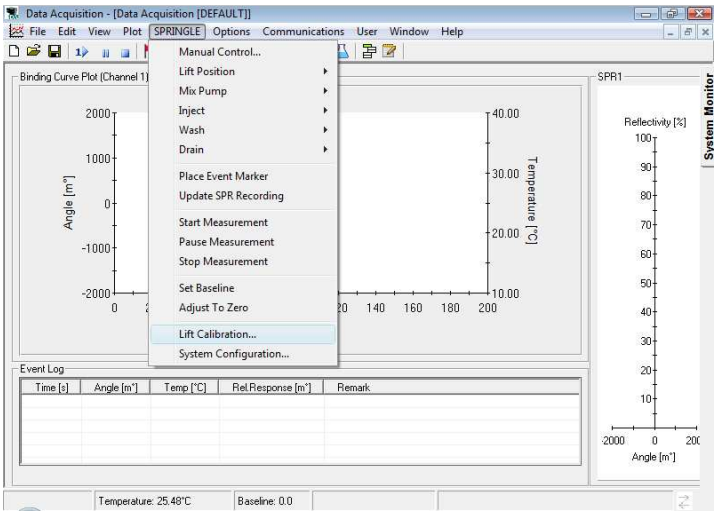


Figure 4.5 – Open the Lift calibration window.

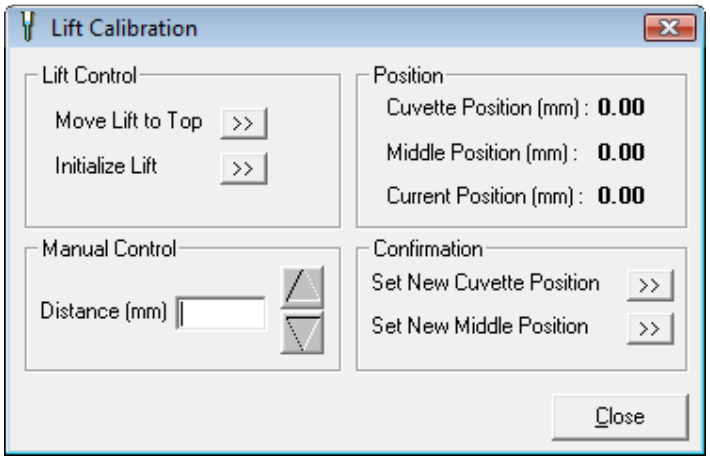


Figure 4.6 – The lift calibration window.

The lift calibration sets the pipette position. The cuvette position for the pipette is normally calibrated to 1 mm above the gold disk; the middle position is calibrated with the pipette just about 2 mm inside the cuvette.

Procedure (specified distances depend on the size of the used pipette tips):

11. Press button 'Initialize lift'.
12. Enter a distance (max. 60 mm). Start with e.g. 50 mm.
13. Press the downward directed arrow button
14. Fine tune distance with 1 mm steps
15. 'Set new cuvette position'.
16. 'Move lift to top'
17. Enter distance of +/- 38 mm and fine tune with 1 mm steps
18. 'Set new middle position'
19. 'Move lift to top'
20. 'Close'.

See Figure 4.7 a and b, step 1 to 10.

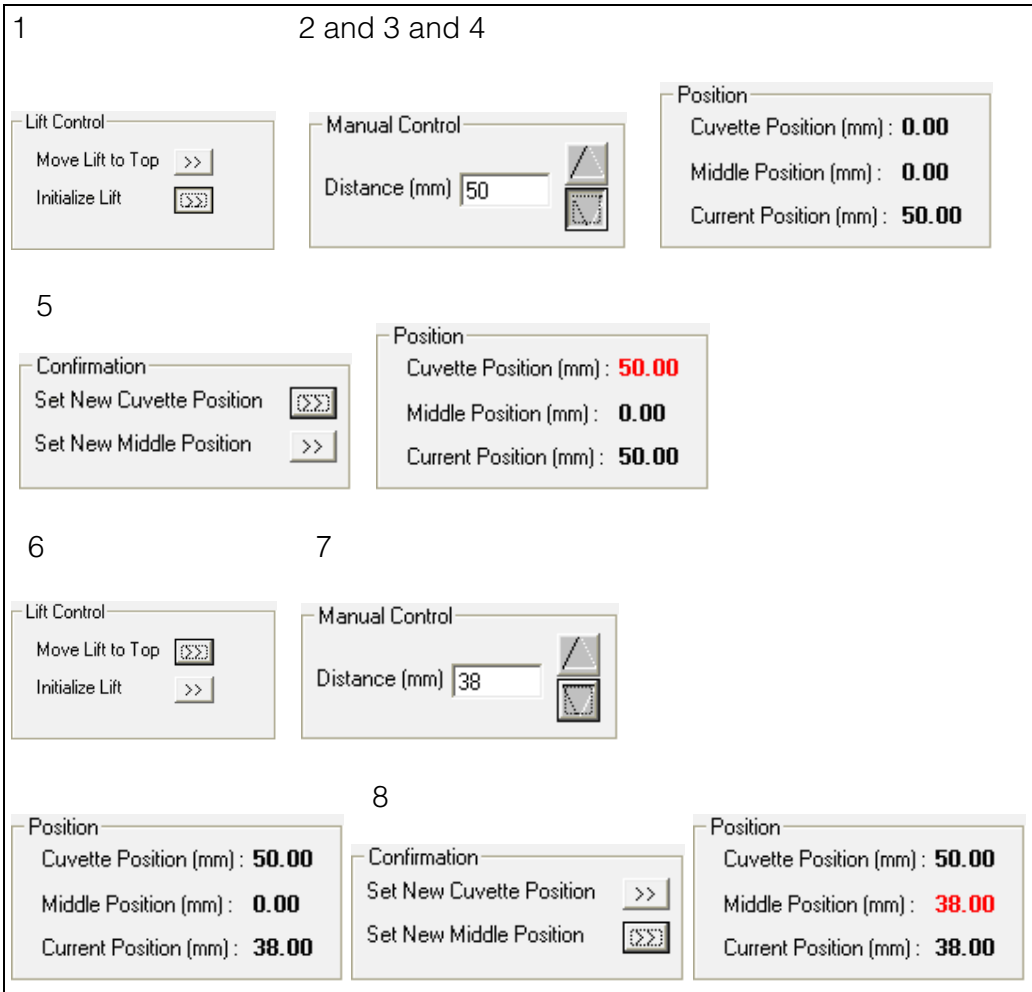


Figure 4.7 – a. The lift calibration procedure.

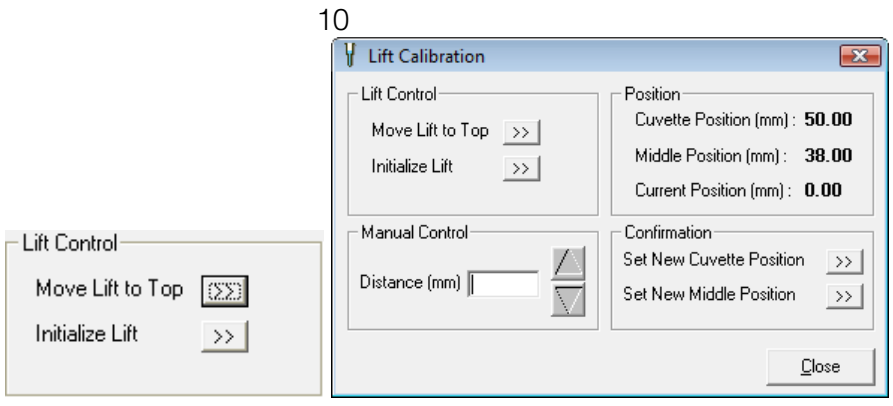


Fig 4.7 – b. The final steps of lift calibration.

4.7 – Installation of the gold sensor disk.

4.7.1 – Preparation of a self assembled monolayer of 11MUA on the gold surface.

Incubate a bare gold disk in a well of a 6 wells culture disk in a solution of 11mg 11-MUA in 50ml ethanol or isopropanol. To get a reproducible quality thiol layer, filter the solution prior to the incubation and perform the incubation overnight. Wash the disk three times with ethanol or isopropanol to remove the excess thiol. To remove the alcohol rinse three times with dematerialized water. Blow the disk dry with compressed air or nitrogen gas. The thiol covered gold disk can be stored dry up to 2 months in the original container.

4.7.2 – Assembling the sensor disk on the hemi-cylinder.

Place a small drop of immersion oil on the outer edge of the hemi-cylinder and gently slide the modified gold disk (coated with 11-MUA) from the start to the end of the hemi-cylinder.

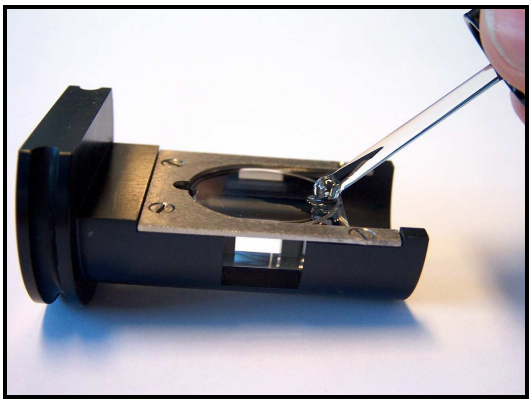


Figure 4.8 – A drop of immersion oil on top of the hemi-cylinder.

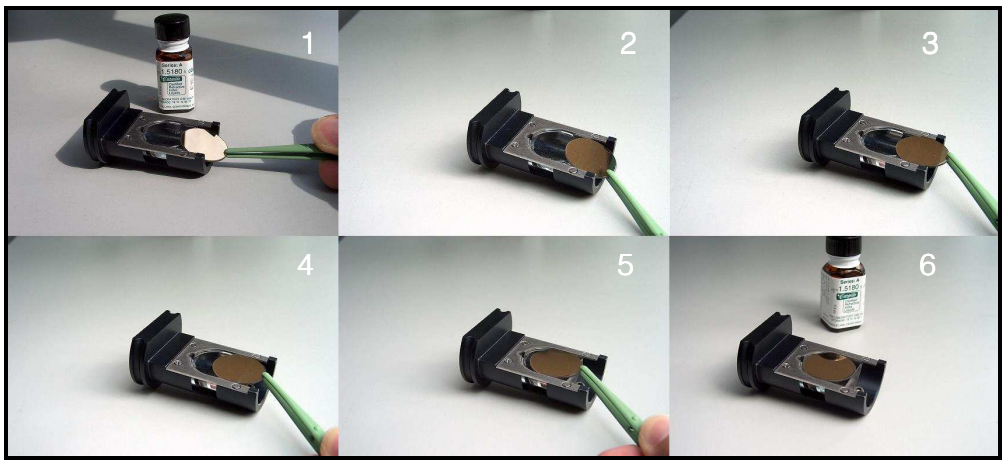


Figure 4.9 – Assembly of a disk.

Be sure that the gold layer is facing up. A rim of gold can be seen at the gold coated side of the disk when slightly tilting it. Make sure that no air bubbles are introduced between the gold disk and the hemi-cylinder. Check this by looking through the hemi-cylinder.

Recommendations;

- Do not manipulate the gold sensor disk with bare hands. We recommend wearing gloves when handling the disk.
- Touch only the frosted sides of the hemi-cylinder to prevent scratches on the accurately polished round sides of the hemi cylinder.
- Manipulate the gold sensor disk with a fine tweezers, especially when you take it out of the plastic case.
- The gold sensor disk in the plastic case is oriented with the gold surface facing down.

Cleaning the hemi-cylinder and slider.

The slider and the hemi-cylinder need to be cleaned regularly because of inevitable spilling of immersion oil: Unscrew the M3x3 screw on the slider. Gently slide the hemi-cylinder out of the slider. Clean the hemi-cylinder with ethanol. Use only the lens tissue paper to clean and dry. Alternatively, the hemi-cylinder and slider can be cleaned in an ultra sonic water bath. After cleaning, slide the hemi-cylinder into the slider with the “oil overflow hole” to the oil overflow hole of the slider. Reassemble the M3 screw.

One Gold disk provides 5 to 7 measuring positions: Set a measuring position by gliding the disk over the hemi-cylinder surface with a clean pipette tip.

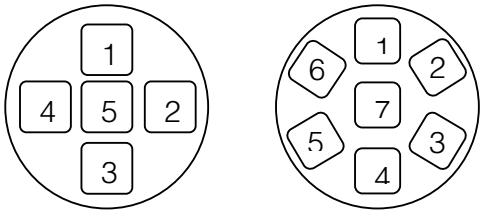


Figure 4.10 – Different positions on the gold disk.

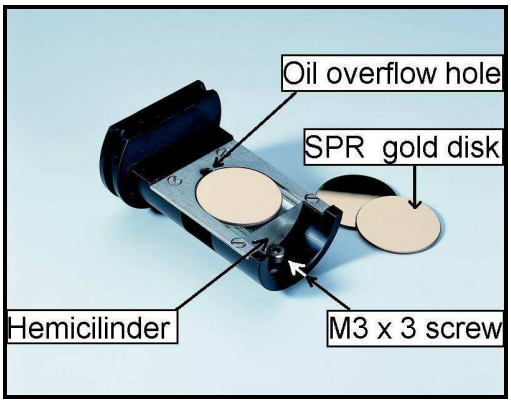


Figure 4.11 – Installed SPR gold disk.

4.7.3 – Installation of the cuvette.

There is only one way to position the cuvette in the cuvette holder. Position the cuvette with the pin towards the slot in the cuvette holder. The cuvette will slide in the cuvette-holder; tighten the cuvette with the ring. Make sure that the ring is tightened firmly to prevent leakage outside the channel. For extra tightening use the supplied SPR key.

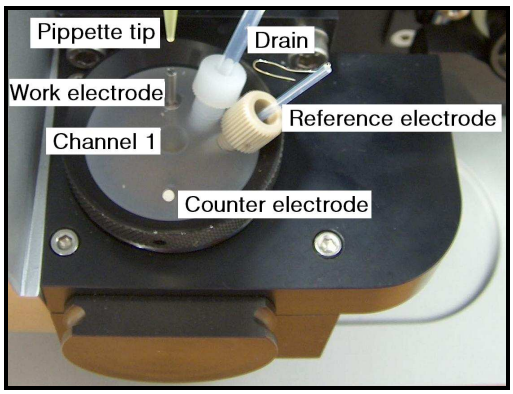


Figure 4.12 – An overview of the cuvette holder.

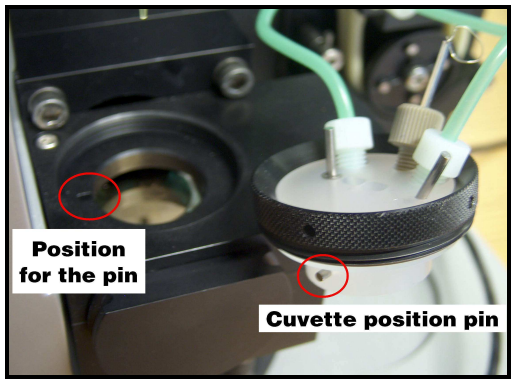


Figure 4.13 –The 'positioning pin' of a cuvette.

4.7.4 – Check for leakage of the measurement channel.

To check for leakage, pipette 30µl PBS into channel 1. Be sure the fluid reaches the gold disk. Check the SPR plot. To monitor the dip continuously, use the scope mode that is available under the Options menu and on the toolbar. Deselect the scope mode by clicking the scope mode button again. If there is no leakage in channel 1, a perfect dip will be seen in channel 1. If the cuvette is not correctly assembled, the dip will change it's form to become more horizontal. Repeat the assembly of the cuvette until it is leakage free.

Awareness of possible leakage!

If there is leakage out of the cuvette onto the hemi-cylinder, the solution may come in contact with the detector. The measurements will become very noisy. A leakage with strong acids may detach the detector out of its calibrated position, causing a hardware problem.

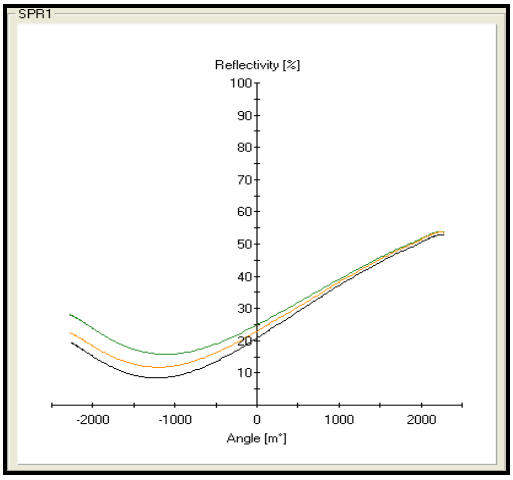


Figure 4.14 – Check for leakage outside of channel 1.

At this point, the sensor disk can be reassembled.

4.7.5 – Fill tubing with buffer/Exchange the buffer solution.

Why should there be liquid in the tubing?

The solution in the tubing is used for washing the pipette tip. Secondly, fluid is not compressible like air, which results in highly accurate sample volumes and flow rates.

In a starting situation, the tubing can be empty (filled with air) or filled with a solution. Run the sequence “initialization of instrument.seq” to fill the tubing with buffer or to change the running buffer as follows:

Select the menu bar item “options” - “Sequencer…”

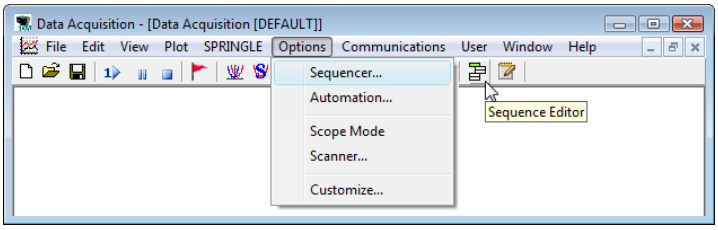


Figure 4.15 –Two ways to activate the Sequencer, via the Menu-Options or the Toolbar button.

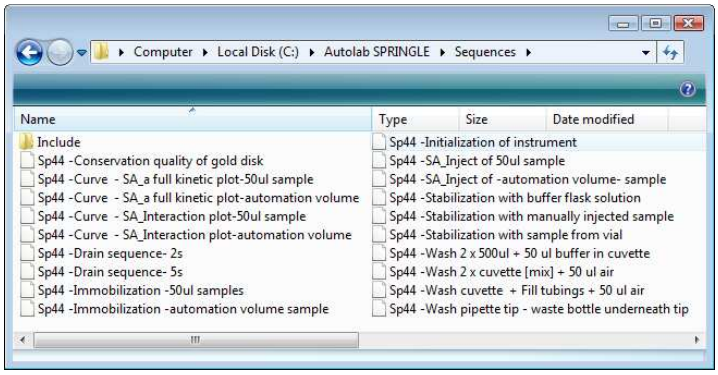

Computer ▸ Local Disk (C:) ▸ Autolab SPRINGLE ▸ Sequences ▸
Name
Type
Size
Date modified
Include
Sp44 -Conservation quality of gold disk
Sp44 -Curve - SA_a full kinetic plot-50ul sample
Sp44 -Curve - SA_a full kinetic plot-automation volume
Sp44 -Curve - SA_Interaction plot-50ul sample
Sp44 -Curve - SA_Interaction plot-automation volume
Sp44 -Drain sequence- 2s
Sp44 -Drain sequence- 5s
Sp44 -Immobilization -50ul samples
Sp44 -Immobilization -automation volume sample
Sp44 -Initialization of instrument
Sp44 -SA_Inject of 50ul sample
Sp44 -SA_Inject of -automation volume- sample
Sp44 -Stabilization with buffer flask solution
Sp44 -Stabilization with manually injected sample
Sp44 -Stabilization with sample from vial
Sp44 -Wash 2 x 500ul + 50 ul buffer in cuvette
Sp44 -Wash 2 x cuvette [mix] + 50 ul air
Sp44 -Wash cuvette + Fill tubings + 50 ul air
Sp44 -Wash pipette tip - waste bottle underneath tip

Figure 4.16 – SPRINGLE; The sequence ‘Initialization of Instrument.SEQ’.

5. Click “sequence” in the menu bar and select “Open sequence”.
6. Select from the list of sequences the file “Initialization of Instrument.SEQ”
7. Press the green arrow toolbar button to run the procedure to fill/exchange the tubing solution with the buffer from the buffer flask.
8. The procedure will flush the liquid handling system, which will give the opportunity to check for leaks at tubing connectors.

At this point the sensor disk can be exchanged. Do not forget to check for leakage after disk exchange.

The instrument is now ready for use; the 11-MUA modified gold disk and the cuvette are assembled correctly, the tubing is filled with the correct buffer.
The measurement can start. The experiments consist of two parts. The first part is to physically attach the BSA protein molecule to the 11-MUA surface. This chemical binding step is called immobilization. The second part is the interaction of the sample anti-BSA antibody with the BSA protein molecule.

4.8 – The immobilization.

4.8.1 – Sample preparation.

For the immobilization procedure, prepare the following samples and solutions:

- Coupling buffer (B4) for baseline and wash steps;
- EDC/NHS activation solution: 60 µl of 1:1 freshly mixed 0.4M EDC (B7) and 0.1 M NHS (B6) in a 1.5ml vial;
- Ligand sample: 200 µl of ligand solution dissolved in coupling buffer (B4) in a 1.5ml vial;
- Deactivation solution: 200 µl of 1M ethanolamine pH 8.5 (B8) in a 1.5ml vial;
- Regeneration solution: 200 µl of 10 mM HCl (B3) in a 1.5ml vial.

4.8.2 – Set angle position of the sodium acetate buffer (B.4).

Put 50 µl of acetate buffer on the gold disk and check the dip by selecting the Scope Mode button . To deactivate the scope mode, press this button again. The scope mode will update the SPR angle every 0.5 seconds.

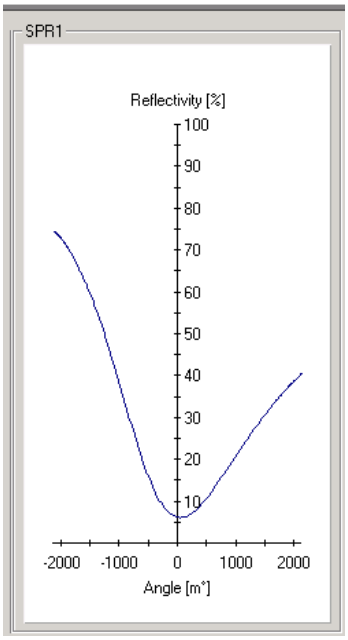


Figure 4.17 – SPR "dip".

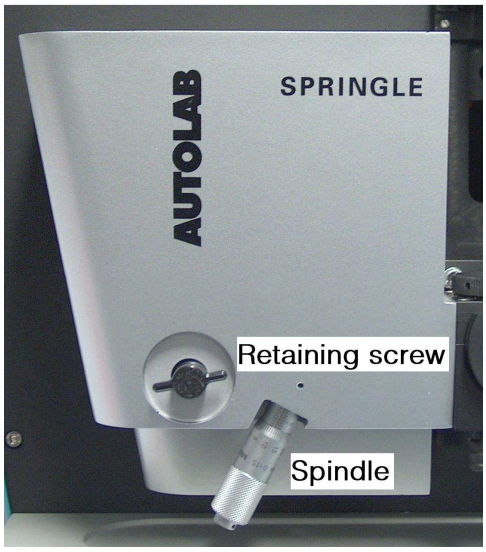


Figure 4.18 – The optical path cover.

Every SPRINGLE instrument is calibrated with water on a bare gold disk. The lowest value of the SPR 'dip' is between 0 and 10 percent absolute reflection.

To change the position of the dip, release the retaining screw of the optical path. Adjusting the position of the dip is done by turning the micrometer spindle. Click on start measurement in the tool bar and follow the change of the angle in real time. Set the baseline around -1500 millidegrees (m°). After adjusting the baseline, fasten the retaining screw again.

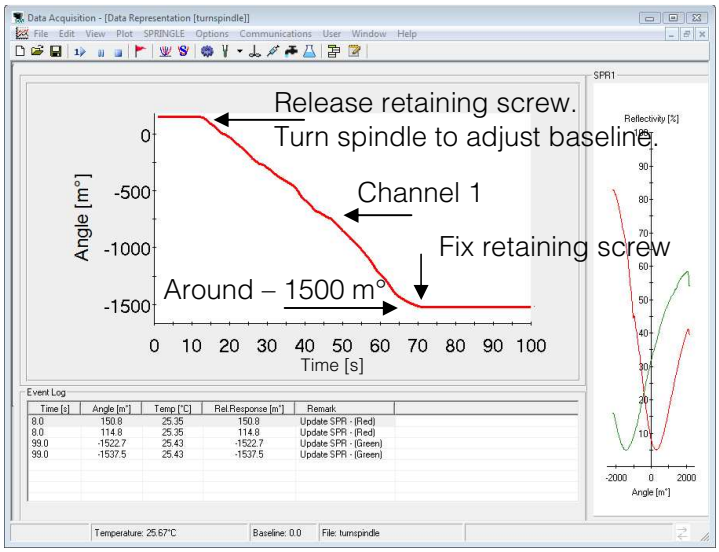


Figure 4.19 – Adjustment of the baseline angle before immobilization.

Why should the sodium acetate buffer SPR angle be set at -1500 m°?

The solutions used in the immobilization differ significantly in refractive index and will change a number of times during the procedure. The acetate buffer has the lowest refractive index and therefore the smallest SPR angle; the Ethanolamine solution has the largest angle.

4.8.3 – Stabilize / rehydrate the dry 11-MUA disk.

Before the modified gold disk can be used for immobilization, the baseline must be stabilized.

Stabilize the surface of the gold disk using one of the sequences:

- Stabilization with buffer from flask.SEQ
- Stabilization with manually injected sample.SEQ
- Stabilization with sample from vial.SEQ

Start the sequence and continue to wash until the baseline is sufficiently stable. The sequence can be stopped at any time by clicking the end measurement button in the tool bar.

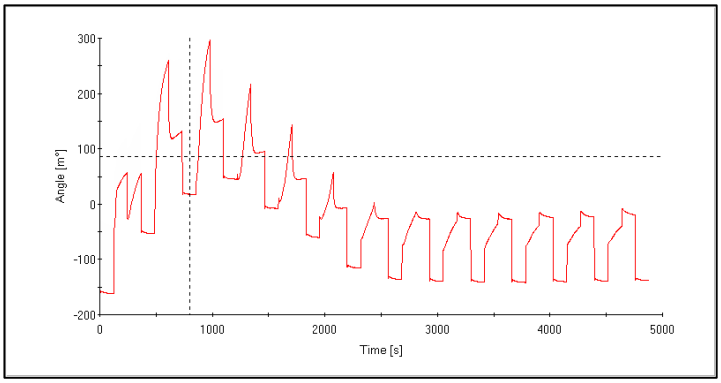


Figure 4.20 – Stabilization/cleaning of the gold disk surface with coupling buffer (B4), regeneration buffer and 0.1 M NaOH.

Eventually, every solution should show the same SPR angle every time is it dispensed on the surface.

Stabilization of the surface is necessary for all kinds of modified gold surfaces!

Commercially available Dextran surfaces need extensive cleaning, like shown above.

4.8.4 – Start the immobilization procedure.

The EDC/NHS immobilization procedure is a standardized procedure that can be easily performed semi-automatically. Open the sequence editor window, and select the file “immobilization.SEQ” .

The lines, 67 (120s), 87 (300s), 141 (900s), 195 (600s) and 249 (300s) are the incubation times for each different step in the immobilization procedure. With a double click on the command line, the settings can be edited.
Select the green button with the arrow to start the immobilization experiment. Whenever an action of the user is required, a message alert will pop up with information to act upon.

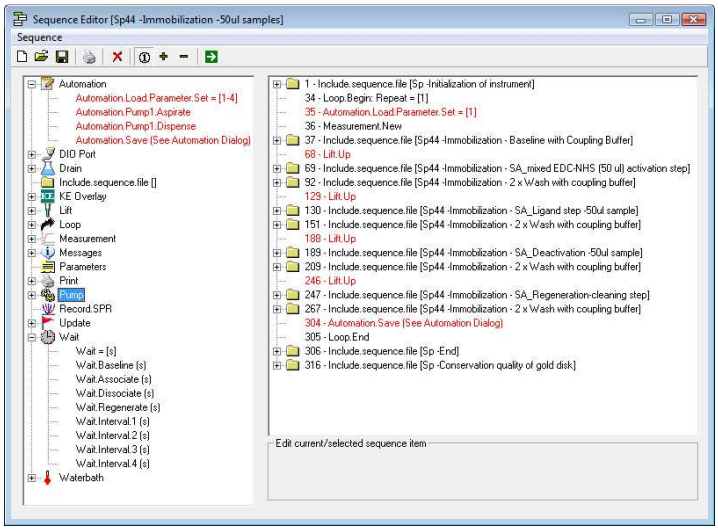

Sequence Editor [Sp44 -Immobilization -50ul samples]
Sequence
Automation
Automation.Load.Parameter.Set = [1-4]
Automation.Pump1.Aspirate
Automation.Pump1.Dispense
Automation.Save (See Automation Dialog)
DIO Port
Drain
Include.sequence.file []
KE Overlay
Lift
Loop
Measurement
Messages
Parameters
Print
Pump
Record.SPR
Update
Wait
Wait = [s]
Wait.Baseline (s)
Wait.Associate (s)
Wait.Dissociate (s)
Wait.Regenerate (s)
Wait.Interval.1 (s)
Wait.Interval.2 (s)
Wait.Interval.3 (s)
Wait.Interval.4 (s)
Waterbath
1 - Include.sequence.file [Sp -Initialization of instrument]
34 - Loop.Begin: Repeat = [1]
35 - Automation.Load.Parameter.Set = [1]
36 - Measurement.New
37 - Include.sequence.file [Sp44 -Immobilization - Baseline with Coupling Buffer]
68 - Lift.Up
69 - Include.sequence.file [Sp44 -Immobilization - SA_mixed EDC-NHS (50 ul) activation step]
92 - Include.sequence.file [Sp44 -Immobilization - 2 x Wash with coupling buffer]
129 - Lift.Up
130 - Include.sequence.file [Sp44 -Immobilization - SA_Ligand step -50ul sample]
151 - Include.sequence.file [Sp44 -Immobilization - 2 x Wash with coupling buffer]
188 - Lift.Up
189 - Include.sequence.file [Sp44 -Immobilization - SA_Deactivation -50ul sample]
209 - Include.sequence.file [Sp44 -Immobilization - 2 x Wash with coupling buffer]
246 - Lift.Up
247 - Include.sequence.file [Sp44 -Immobilization - SA_Regeneration-cleaning step]
267 - Include.sequence.file [Sp44 -Immobilization - 2 x Wash with coupling buffer]
304 - Automation.Save (See Automation Dialog)
305 - Loop.End
306 - Include.sequence.file [Sp -End]
316 - Include.sequence.file [Sp -Conservation quality of gold disk]
Edit current/selected sequence item

Figure 4.21 – The sequence editor showing the sequence "immobilization.SEQ".

4.9 – The interaction.

4.9.1 – Sample preparation.

For the interaction procedure, prepare the following samples and solutions:

- Running buffer, PBS (B2) for baseline, wash steps and dissociation;
- Anti-BSA dilutions for association (B9; B10; B11): 60 µl in a 1.5ml vial;
- Regeneration solution: 60 µl of 10 mM HCl (B3) in a 1.5ml vial.

4.9.2 – Stabilize the surface.

Change the coupling buffer in the flask with PBS (B2). Stabilize the surface as described in section 4.8.3 of the immobilization paragraph. Thiol layers, Dextran layers or surfaces with immobilized ligands have to be stabilized to minimize matrix effects that are caused by differences in pH or ionic strength (high-low salt concentrations) of the different buffers used throughout the experiment. The matrix effects influence the SPR signal. Due to exposure of the layer with the different buffers of the experiment, the layer will respond in a more predictive way and will continuously give a SPR signal at the same angle. When the desired stability is reached, the sequence can be stopped at any time by clicking the "Stop measurement" button in the tool bar.

4.9.3 – Start the association procedure.

The experimental association procedure is a standardized procedure that can be easily performed semi-automatically. Open the sequence editor

window, and select the file “Curve - SA – a full kinetic plot.SEQ”.The lines, 67 (120s), 86 (3600s), 117 (600s), 146 (300s) and 178 (120s) are the incubation times for each different step in the full kinetic plot procedure. With a double click on the command line, the settings can be edited.

Select the green button with the arrow to start the experiment. Whenever an action of the user is required, a message alert will pop up with information to act upon.

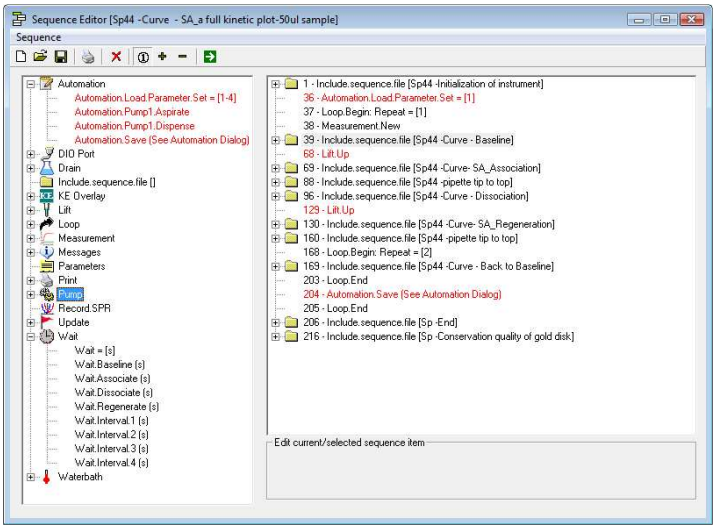


Figure 4.22 – Sequence Editor Window showing the “curve – AS- a full kinetic plot.SEQ” sequence.

4.10 – The SPRINGLE Data.

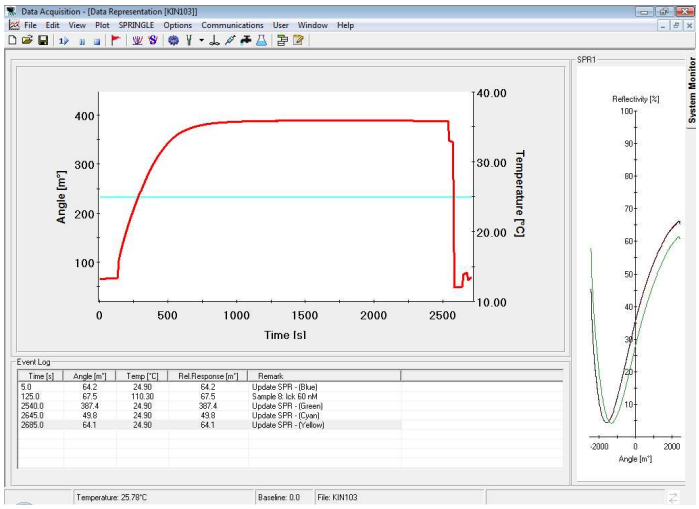


Figure 4.23 – A typical example of an association experiment.

4.11 – Cleaning of the SPRINGLE instrument.

This is a guiding principle for cleaning all part's in the system which are in contact with the solutions used in the experiments. Replace the buffer flask solution step by step with cleaning solution after the specific sequence is finished.

Cleaning Solution 1: - 0.5% (w/v) SDS/ 1% (w/v) Triton in water
Total cleaning time about 10 min.

Cleaning Solution 2: - 0.5% (w/v) SDS
Total cleaning time about 10 min.

Cleaning Solution 3: - 50 mM Glycine-NaOH pH 9.5
Total cleaning time about 10 min.

Cleaning solution 4: - 6 M Urea
Total cleaning time about 10 min.

Cleaning solution 5: - 1% acetic acid
Total cleaning time about 20 min.

Cleaning solution 6: - 0.2 M NaHCO3
Total cleaning time about 10 min.

Cleaning solution 7: - Hydrochloric acid : 0.1 M HCl
Total cleaning time about 20 min.

Cleaning solution 8: - Water
Total cleaning time about 10 min.

Cleaning solution 9: - 70% Ethanol
Total cleaning time about 10 min.

A. Clean before shutting down for a weekend;
    - Use the sequence "*; 'Initialization of Instrument.SEQ'*
    - Put the inlet buffer flask tubings out off the flask
    - Run the sequence to empty all tubings
    - It's also OK to replace the solution in the tubings with Solution 7, water
B. Clean needles, cuvette and connected tubings once every two weeks
    - Use the sequence "*; 'Initialization of Instrument.SEQ'*
    - Place all inlet tubings into the 'buffer' flask
    - Run the sequence to clean with solution 1 and 8
C. Total clean of the system every two month's

- Use the sequence "*; 'Initialization of Instrument.SEQ'*
- Place all inlet tubings into the 'buffer' flask
- Run the sequence using every solution 1, 3, 7, 8, 9 step by step

D. Total clean of the system every half year

- Use the sequence "*; 'Initialization of Instrument.SEQ'*
- Place all inlet tubings into the 'buffer' flask
- Run the sequence using every solution 2, 4, 5, 6, 8, step by step

Use the routine *'Initialization of Instrument.SEQ'* to prepare the system before use.

Running buffer 1: PBS pH7.4

Or Running buffer 2: 10 mM HEPES, 150 mM NaCl, 3.4 mM EDTA, 0.005% Tween P20

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Chapter 5

5 – Data Acquisition software.

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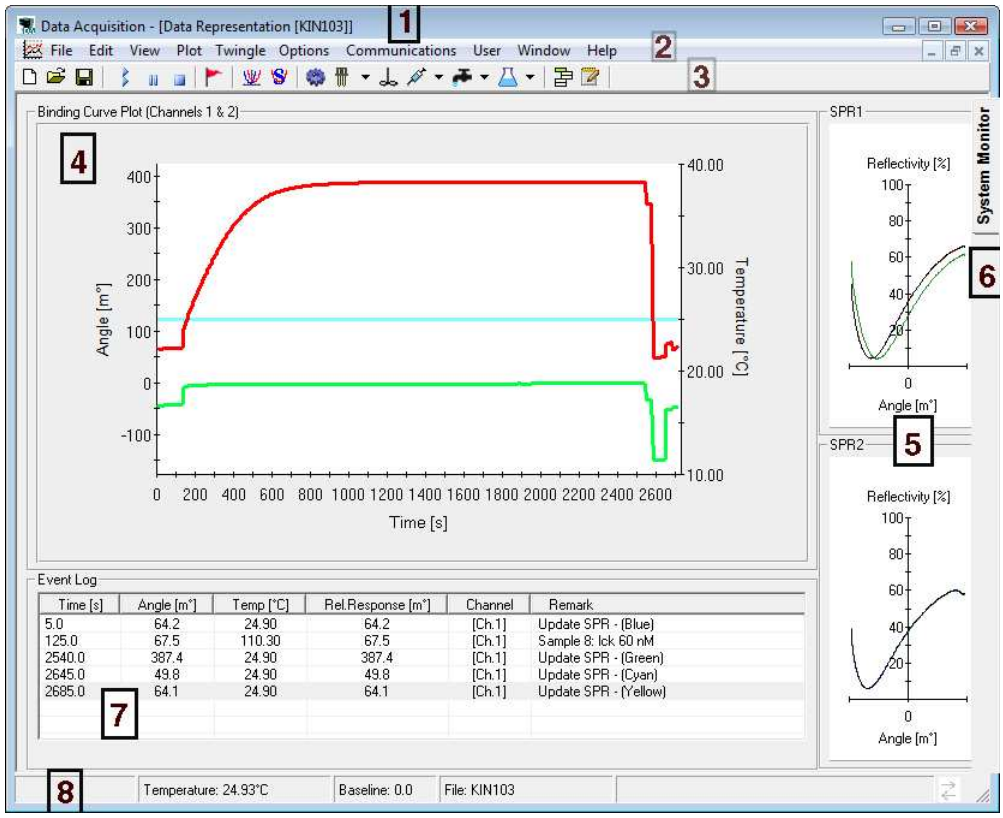


Figure 5.1 – Data Acquisition software.

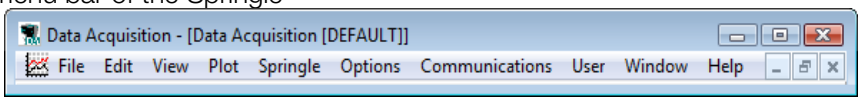
The screen contains top -bottom the Title bar (1), Menu bar (2), Tool bar (3), Binding Curve Plot (4), SPR 1 and 2 Plot (5), System Monitor bar (6), Event Log (7) and Status Bar (8).
This chapter will provide in-depth coverage of the TWINGLE/SPRINGLE software.

5.2 – Overview of the functions.

The Menus

A short overview of the menus is given below.
The functions for all menu items will be explained in the next sections.

A. The menu bar of the Springle



B. The menu bar of the Twingle.

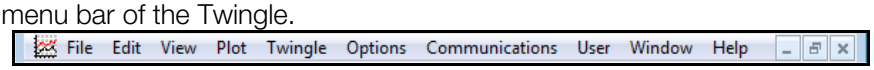
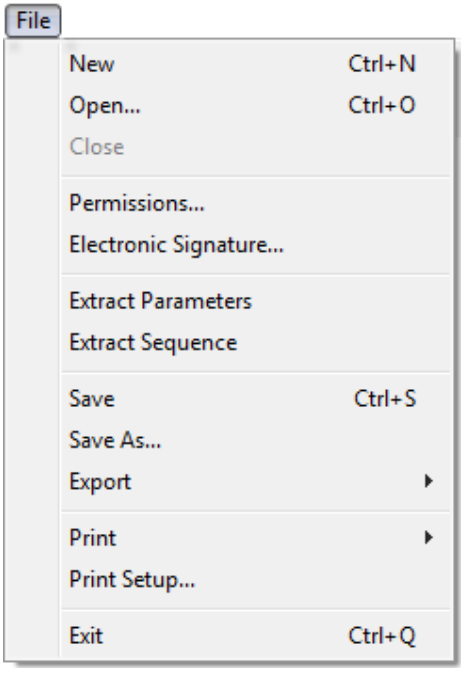
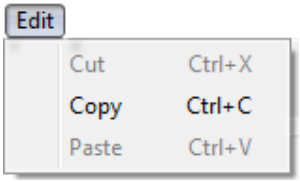


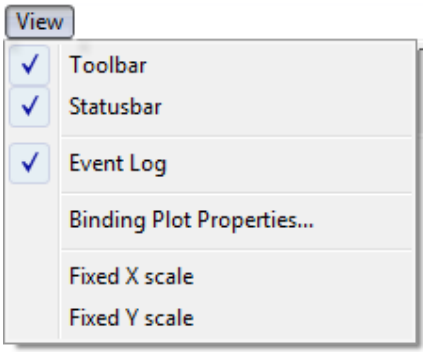
Figure 5.2 – The Data Acquisition menu bar.



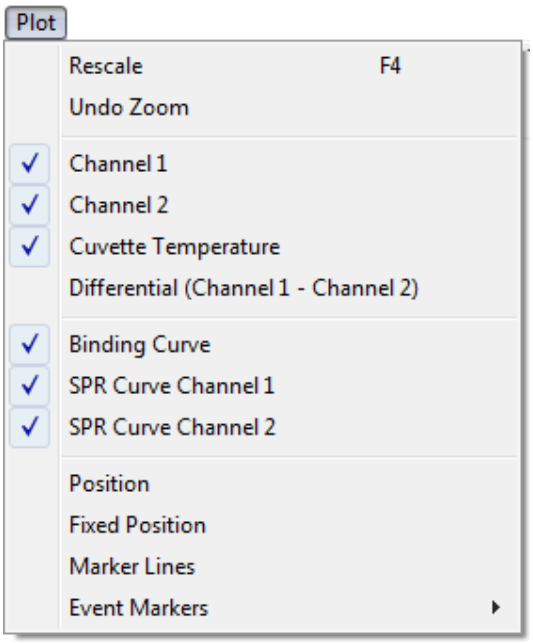
File menu, shows all the instructions related to old and new experimental data files, like open, save, export and print a data file. Some items are only available in the security software version. **Note**: some of the instructions have a corresponding button that is present in the toolbar. Two commands are only visible in the security software version.



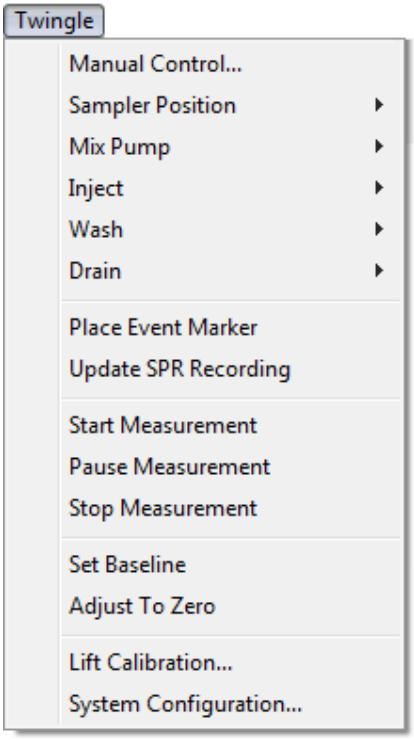
Edit menu, shows all the edit-related commands.



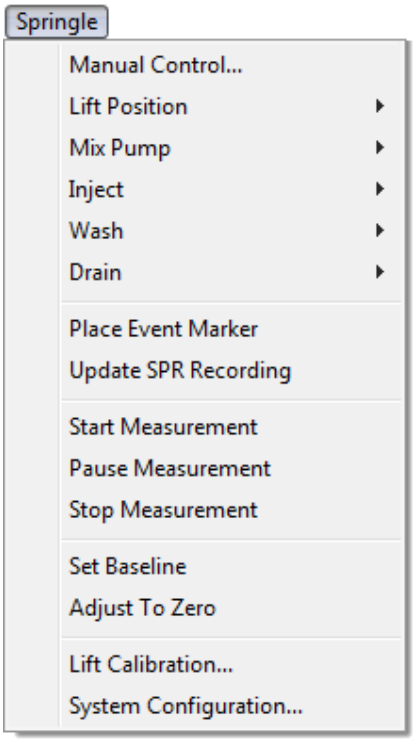
View menu, shows all the view-related commands. **Note**: some of the instructions have a corresponding command using the right click with the mouse.



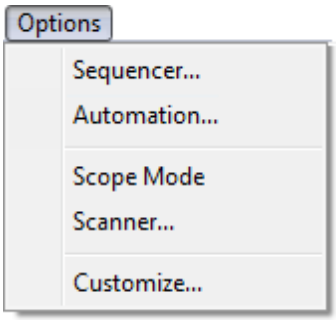
Plot menu, shows all possibilities to organize the data acquisition window plots. **Note**: some of the instructions have a corresponding command using the right click with the mouse.



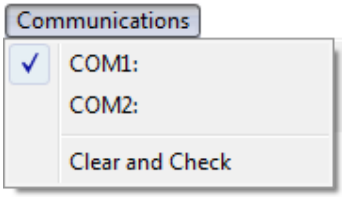
TWINGLE menu, shows all commands to control the instrument and the experiment. . **Note**: some of the instructions have a corresponding button that is present in the toolbar. Calibration only when the pipette tip is replaced.



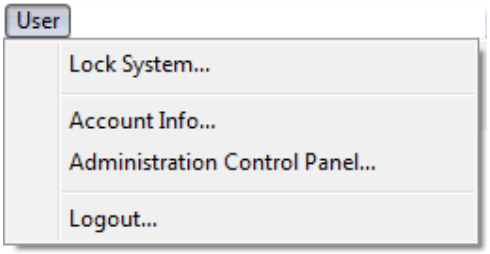
SPRINGLE menu, shows all commands to control the instrument and the experiment. . **Note**: some of the instructions have a corresponding button that is present in the toolbar. Calibration only when the pipette tip is replaced.



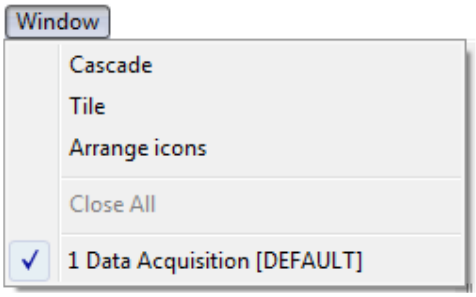
Options menu, used to choose personal settings, execution of sequence, or control of the scanner.



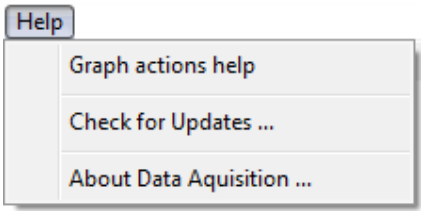
Communications menu, used to select the right COM port or reconnect to the instrument.



User menu, used to control the accessibility of users. **Note**: This menu is only available after installing the security version.



Window menu, used to organize multiple data acquisition windows.



Help menu, used to check the software version.

The toolbar buttons.

Clicking a specific button in the toolbar can perform most of the manual handling for measuring SPR. Figure 5.3 shows an overview of the DA toolbar and its buttons.

A. The toolbar of the Springle

B. The toolbar of the Twingle

Figure 5.3 – The Data Acquisition tool bar.

The toolbar shows a number of buttons, some of which might be grayed out (in which case the attached instruction cannot be performed).

This section provides an overview of the toolbar buttons.

New , Opens a new data acquisition window for a new measurement.

Open, SPR data files [*.ibo (old software versions) or *.spr] in a new window.

Save, saves (or saves as) the currently measured SPR data.

Start measurement, starts the measurement

Pause measurement, pauses the measurement

Stop measurement, stops recording the measurement.

Place Event Marker, Add a marker in a measurement plot

Update SPR recording, Records a SPR signal, 'dip', during the measurement.

Scope mode, Refreshing update SPR every 0.5 s.

Manual control, Manual measurement

Sampler position, To control the position of the lift.

Mix: Customize information window pops up. Press the arrow and a selection of three different sequences can be made.

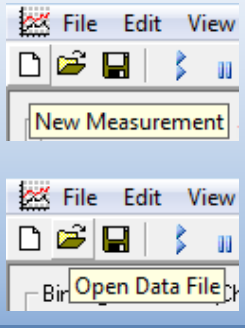
Inject: A selection of Press the arrow and a selection of three different sequences can be made.

Wash: (de-) activates wash peristaltic pump.

Drain: (de-) activates drain peristaltic pump.

Sequence editor; The SPR sequence editor is used to automate measurements, to create, adjust and to execute sequences.

Automation: The Automation window handles the experimental setup, parameters, incubation times, and the sequences to automate an experiment.



The tooltip

Hovering your mouse pointer over one of the buttons will trigger a Tooltip to appear, displaying some basic information on the functions of that button.

5.3 – File menu.

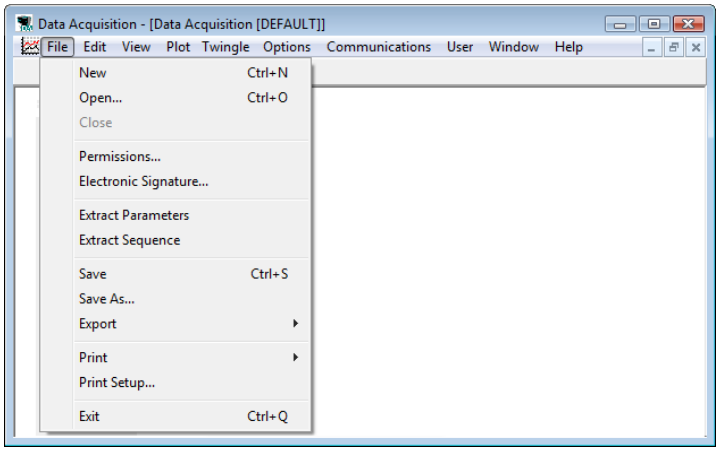


Figure 5.4 – File menu.

New

Opens a new data acquisition window for a new measurement.

Open

Opens a data (*.spr or *.ibo) file window.
The *.spr file will have all data information in one file; the SPR measurement plot (max. 50,000 data points), the eventlog, the SPR signal (max. 20), the experiment parameters, and the sequence.
The *.ibo file, an old software version used extension, is linked to the *.ini, *.spo, and *.spe file extensions. These file type are measurements performed with software version 4.1 and sooner.

- Data file (*.ibo) for a data acquisition plot.
- Procedure file (*.ini) for measurement settings.
- SPR file (*.spo) for SPR curves of channel 1 (12 maximum).
- SPR file (*.spe) for SPR curves of channel 2 (12 maximum)

(Only the double channel will have the *.spe file).

Close Closes a data acquisition window without saving.

Permissions Allows the user to specify the level of visibility for their files. This is explained in the Autolab SPR system security manual.

Electronic Signature
Allows users to electronically sign files. This is explained in the Autolab SPR system security manual.

Extract Parameters
Opens the manual control window showing the used parameters of the stored measurement.

Extract sequences
Opens the sequence editor showing the sequence used in the stored measurement..

Save

Saves a data acquisition window as data file (*.spr) under the current name. Previously saved files with the same name will be overwritten.
Remark: The default directory to store the data needs to be specified in menu Options - Customize settings – User directories, see Figure 5.26.

Save as Opens a ‘save as’ window to save recorded data as data file, with a user created filename and directory.

Export Export data as a text file (which can be imported in Excel for instance) or export graphical plots as a BMP picture file.

Print Print plot:
- Binding Curve Plot; a data acquisition plot: SPR angle vs. time.
- SPR Plots; intensity of the reflected light vs. angle.
- Event Log; event log file.

Print Setup Opens the print set-up window for selection of printer and printer settings.

Exit Exits TWINGLE program and saves the current settings as default parameters.

5.4 – Edit menu.

Cut Deletes the selected region of a text.

Copy Copies the selected object to the clipboard. The copied object can be retrieved in Microsoft Word or Excel with the paste command.

Paste Copies the clipboard contents into the current selected object.

5.5 – View menu.

The view menu contains commands for presentation of data acquisition plots.

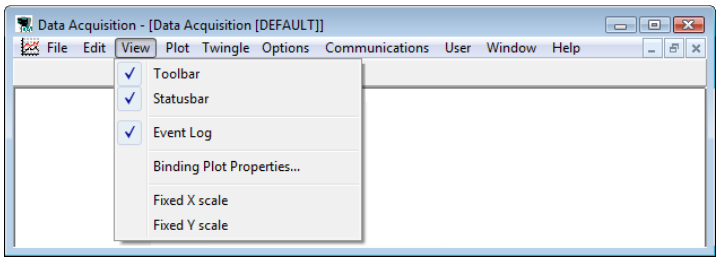


Figure 5.5 – View menu.

Tool bar Option for opening or closing the tool bar.

Status bar Option for opening or closing the status bar.

Event log Option for opening or closing the event log. The event recorder window records important events from the data acquisition plot. Events such as an SPR update or a manually set event marker are recorded here. Events can be edited by double clicking the text entry in the log.

Fixed X scale Fixes the current X-axis scale during the measurement.

Fixed Y scale Fixes the current Y-axis scale during the measurement.

Binding plot properties
Possibility to change the curve and/or graph settings.

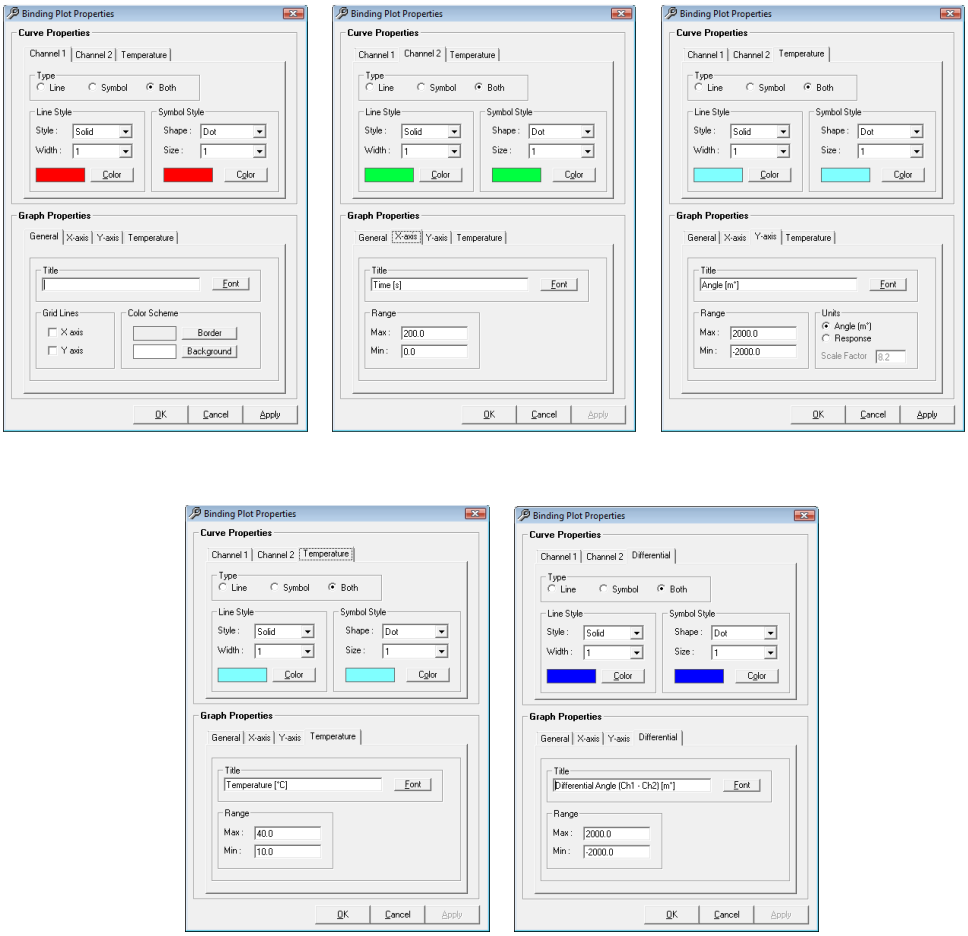
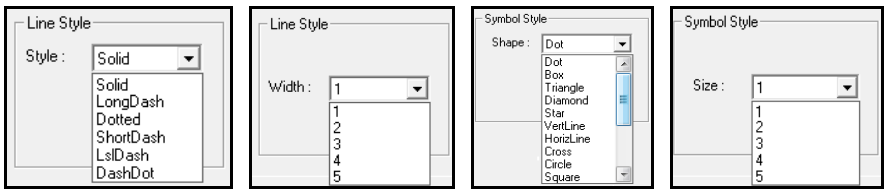


Figure 5.6 – The different tab sheets to adjust the curve or graph properties.

The options to change the curve are based on curve style, width, size or color. The graph changes are text, font, color, change of axis unit, grid lines or axis range.



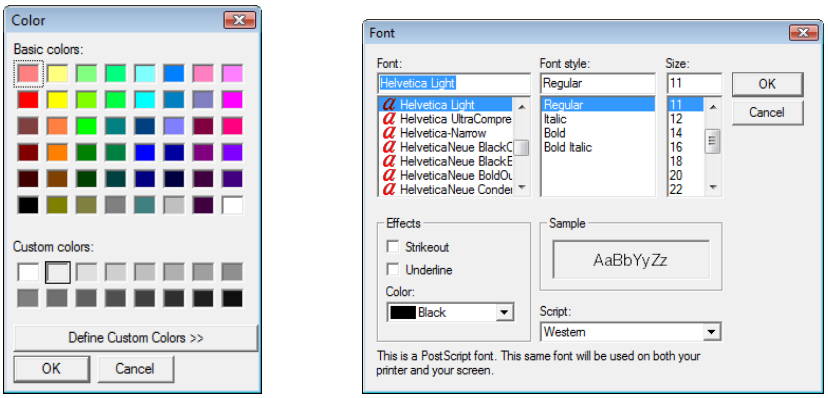


Figure 5.7 – The options of adjusting the curve or graph properties.

The ‘view’ options of Data Plot Properties is available with the mouse right click. (See picture 5.9)

Zoom: Use the left mouse button to zoom in on the plot. The available ‘unzoom’ option (see section 5.6) can unzoom up to 30 zoom actions.

Move: press Shift and hold down both mouse buttons (or middle button on 3-button mouse). Move the mouse to change the positioning of the chart.

Reset: Press the F4 function key to remove all scaling, moving and zooming effects. The “r” key is used to remove all scaling effects in the active plot (DA, SPR1 or SPR2).

View: Right mouse click on the DA window to open a window to change the layout of all components. The window contains plot menu options and view menu options. Extra options are the Curve and Graph Properties window. This window allows to change axis scale for all data lines like; angle, time, temperature and differential. It is also possible to put a title on top of the graph, set grid lines and change the borderline and background colour. See Figure 5.7 and section 5.6.

Depending on the second Y-axis choice (temperature or differential), opening the plot-view option list with a left mouse click, the last tab on the Graph properties window will be either differential or temperature.

Within the Curve Properties window, all data line properties can be changed, see Figure 5.6.

5.6 – Plot menu

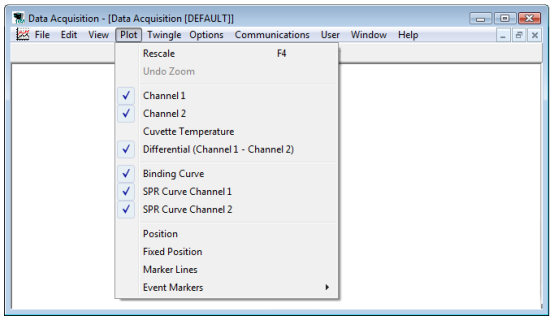


Figure 5.8 – Plot menu.

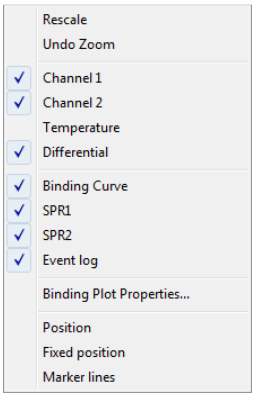


Figure 5.9 – Right mouse click in DA window.

This menu contains options to adjust the Data Acquisition plot and the SPR plot. All items can also be controlled with a right mouse click in the Data Acquisition window (see Figure 5.9).

Rescale Undo zoom function, also possible with key “r” (case sensitive). This will remove all scaling, moving and zooming effects.

Undo Zoom With the ‘left mouse click’ an area can be zoomed in, up to 30 zoom levels can be restored.

Channel 1 Shows or hides data-line of channel 1 in the data acquisition window.

Channel 2 Shows or hides data-line of channel 2 in the data acquisition window. (Twingle only)

Differential (channel 1 – channel 2) (Twingle only)
Shows or hides the differential data of channel 1 minus channel 2.
When this option is activated, it is not possible to view the temperature as the second y-axis.

Cuvette temperature
Shows or hides a second y-axis with temperature scale. The temperature is always measured, even when the temperature plot is not visible in the data acquisition plot.

Binding curve Shows or hides the data acquisition plot, angle versus time.

SPR curve channel 1

Shows or hides a SPR plot of intensity versus of angle of channel 1,

SPR curve channel 2

Shows or hides a SPR plot of intensity versus of angle of channel 2. (Twingle only)

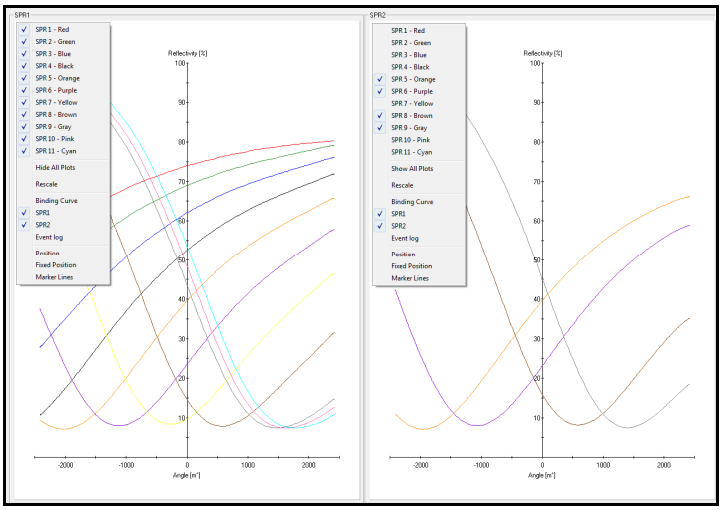


Figure 5.10 – SPR curves of channel 1 and SPR curves of channel 2
With a right mouse click on the SPR plot window, the user can select or deselect a recorded SPR curve. In this example, four SPR updates are still visible in the Channel 2 SPR plot.

Position Shows or hides x and y positions of the mouse in the upper left corner of the data acquisition window.

Fixed position Connects the marker lines with channel 1 or channel 2 data points depending on the closest position of the mouse to a curve.

Marker lines Option to show or hide a flexible x and y axis as a mouse pointer, this enables to read the interception values of a data point on the axes.

Event Markers Shows or hides the markers of channel 1 and/or channel 2 in the data acquisition window.

5.7 – TWINGLE/SPRINGLE menu.

The TWINGLE/SPRINGLE menu contains commands to control the hardware functions of the instrument.

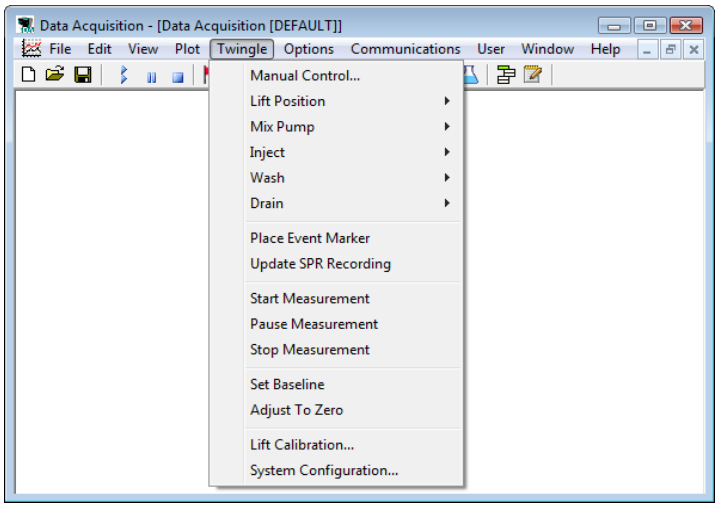


Figure 5.11 – TWINGLE menu items.
The SPRINGLE has the same list of items.

5.7.1 – Manual Control of the Autolab.

Opens the pump control window used for manual control of a measurement.

Sample identification

The Sample identification text box can be used to specify the name and the concentration of the sample. Specified text will be shown in the event log when a measurement is started.

Lift position

Move the lift up and down. 'Down' is the inject position in the cuvette, 'middle' position is just inside the cuvette and the 'up' position is the home position. All positions can be calibrated in the lift calibration window. After the installation of the software, the lift position must be calibrated.

Measurement settings

Measurement Settings box item 'Interval time' allows the user to choose the time between two data points. Interval times between 0.1 and 300 seconds are possible. Adjust to zero time (between 1 to 10 seconds) can be used to average the data for calculating the offset to adjust to zero.

Pump 1 and 2 are syringe pumps (Springle has only pump 1)

Aspirate/Dispense section to aspirate or dispense sample in or out of the cuvette, the sample can be positioned in a microtiter plate or a vial which is held manually under the needles.
Valve options control a three-way valve: valve to needle connects channel from pump to cuvette. Valve to buffer connects channel from buffer flask to pump.
Flow control enables to select the speed of the pump in µl/s. Corresponds to the aspirating, dispensing and mixing speed.
Mix section for mixing the solution in the cuvette. Fill in the mixing volume in µl and check the mix box to start mixing. This option should only be used when the needles are inside the cuvette and the mix volume should not exceed the cuvette volume.

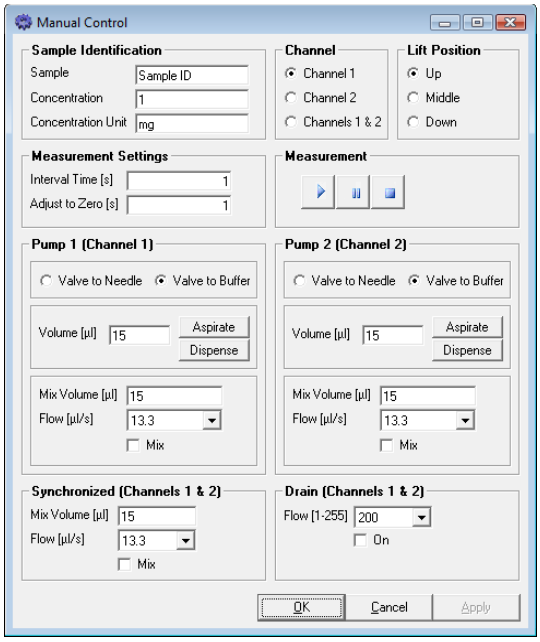


Figure 5.12 A. – Manual control window of the Twingle.

Synchronized Pumps (Channels 1 & 2) (Twingle only)

This section is meant for synchronized mixing of pump1 and pump2. Set volume and Flow, check the mix box and both pumps will start mixing at the same time, with the same flow and with the same volume.

Measurement box

The buttons to start, to pause or to stop the measurement.

Drain is a peristaltic pump

Select the pump speed and check the “on” box to activate the pump, the solution is drained from the cuvette.

Remark: In the software the peristaltic pumps are listed as having a selectable range from 1 – 255. In fact, the peristaltic pump has a maximum flow at 40 rpm selectable in 255 steps.

The SPRINGLE manual control has one extra item the “Continuous Flow”

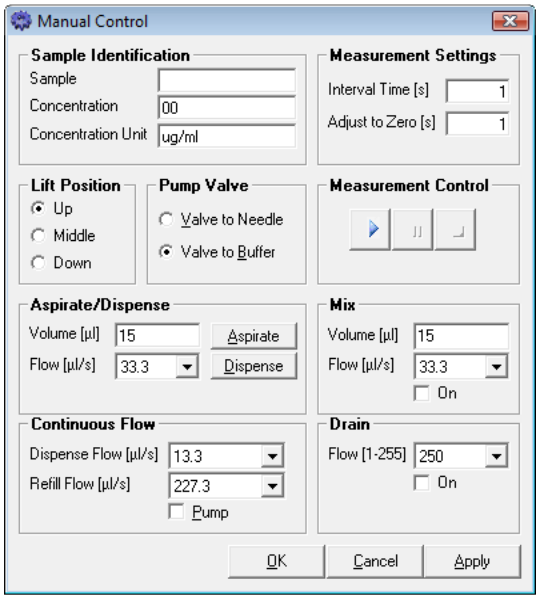


Figure 5.12 B. – Manual control window of the SPRINGLE.

Continuous Flow

The command item will dispense the syringe solution into the pipette tip continuously. During refill, the pump valve is automatically set to buffer flask and the piston will go its lowest position to aspirate buffer from the flask. Then the valve is set to pipette tip to dispense the buffer.

5.7.2 – Lift position in the software.

Up position Home position of the lift.

Middle position Calibrated position in the lift calibration window. Normally set at a few millimetres inside the cuvette

Down position Inject position, calibrated in the lift calibration window. Normally set at 1 mm above the gold disk.

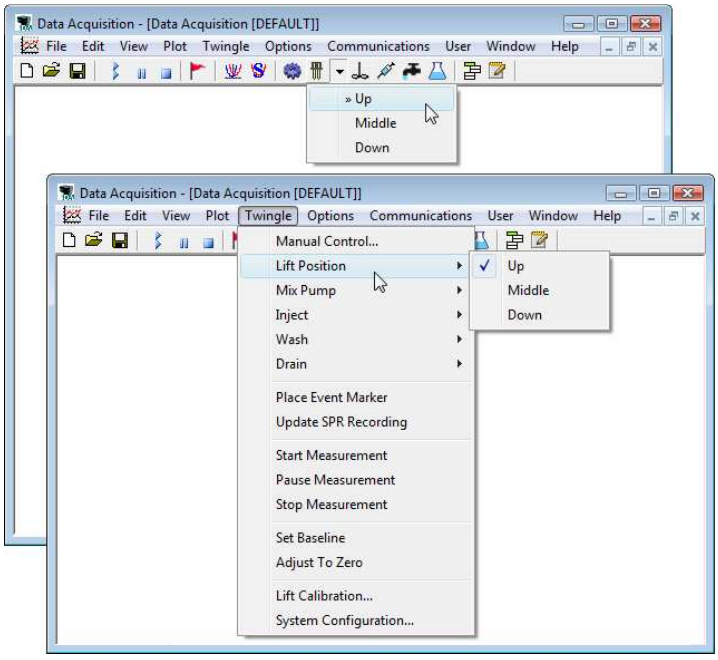


Figure 5.13 – Example of two TWINGLE DA screens with Lift Positions choices via Menu bar or Toolbar.
SPRINGLE has equal functionality.

5.7.3 – Inject

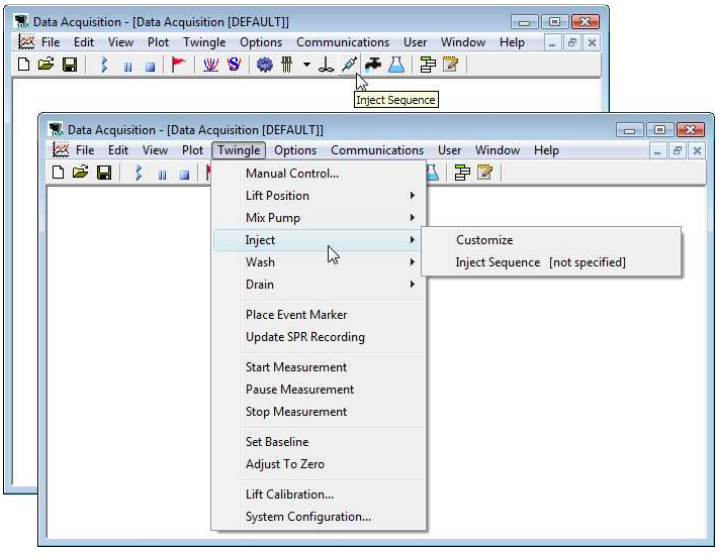


Figure 5.14 – Two TWINGLE DA screens showing two ways to get quick access to customize and to sequences.
SPRINGLE has equal functionality.

Press the toolbar inject picture and the Customize information window pops up.

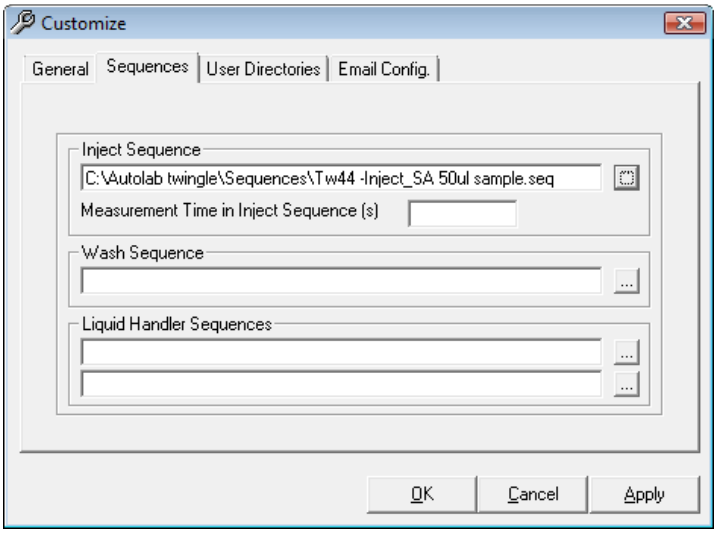


Figure 5.15 – Example of a Customize window to change a linked sequence shown in the Menu_Twingle "Inject". SPRINGLE has equal functionality.

The Customize window is needed for selection of a sequence that will be linked. Select Customize and the customize window will pop up. On the tab page 'Sequences' a link can be established to the inject sequence icon in the Menu_Twingle "Inject". (see Figure 5.15).

Using the menu TWINGLE "Inject", the name of the sequence which could be linked would be replacing the words "not verified"; "Inject Sequence [not verified]".

Using a measurement sequence, the 'Measurement time in inject sequences[s]' (incubation time) shown in the customize window will overwrite the incubation time command "wait" in the sequence. (see Figure 5.16)

The flexibility of the customize window is the ability to link all other kinds of sequences besides inject sequences to the inject toolbar arrow.

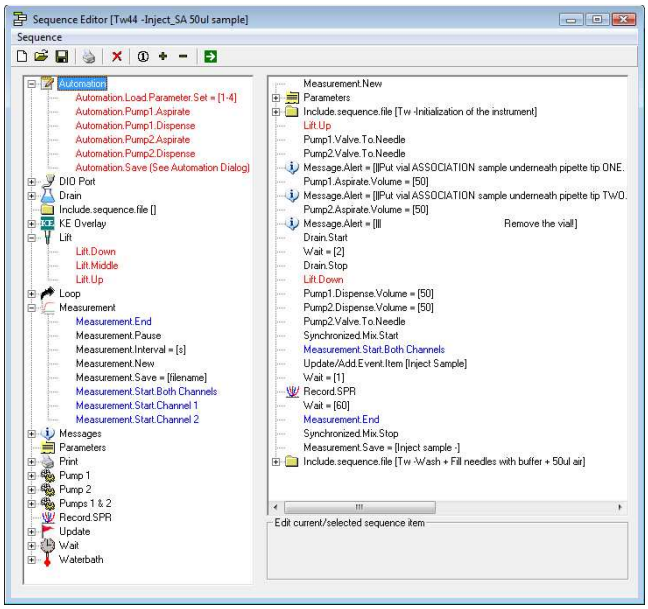


Figure 5.16 – An example of a TWINGLE inject sequence. However, Line 70, 72 has a defined incubation time, which will be overwritten by the time shown in the Customize Sequence tab sheet (Fig 5.15).

5.7.4 – Wash.

Press the toolbar WASH icon and the Customize information window pops up. (see Figure 5.18). Via the Menu TWINGLE “Wash”, a “user-defined sequence” can be defined to be executed or choose to open the Customize window.

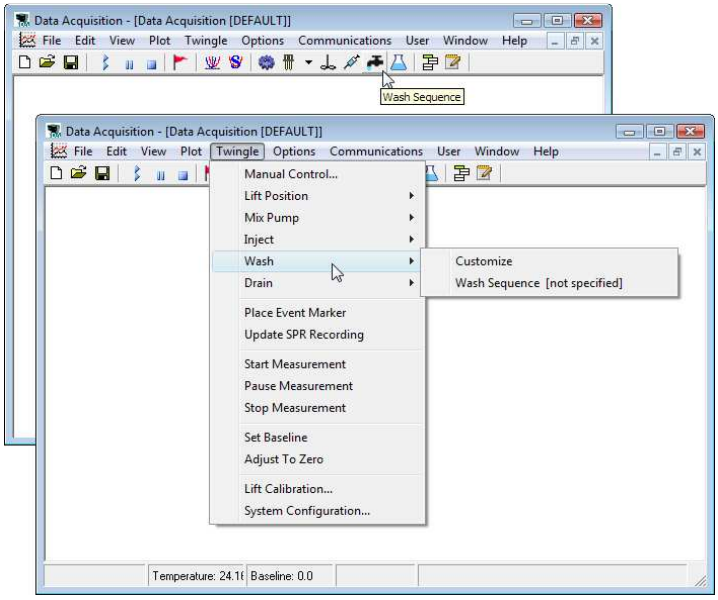


Figure 5.17 – Two TWINGLE DA screens showing two ways to be able to get quick access to customize and to sequences. SPRINGLE has equal functionality.

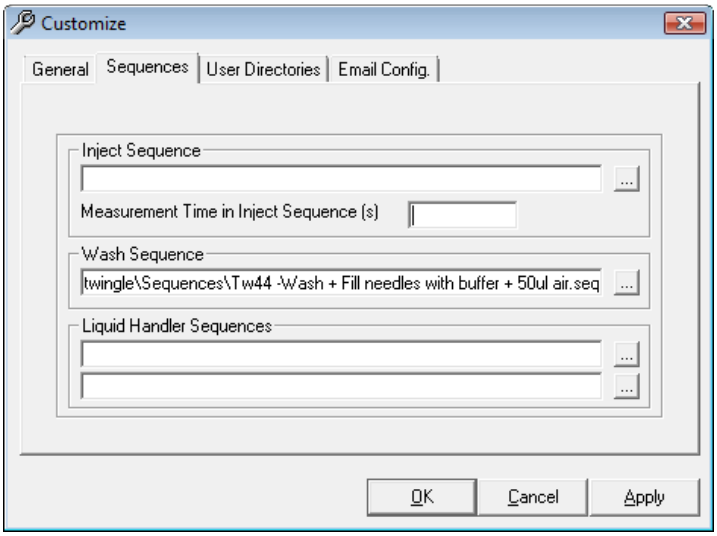


Figure 5.18 – Example of the Customize window to change a linked sequence shown in the Menu_Twingle “Wash”. SPRINGLE has equal functionality.

5.7.5 – Drain.

The DRAIN button in the toolbar will switch ON or OFF the peristaltic drain pump. The speed is defined in the manual control window.

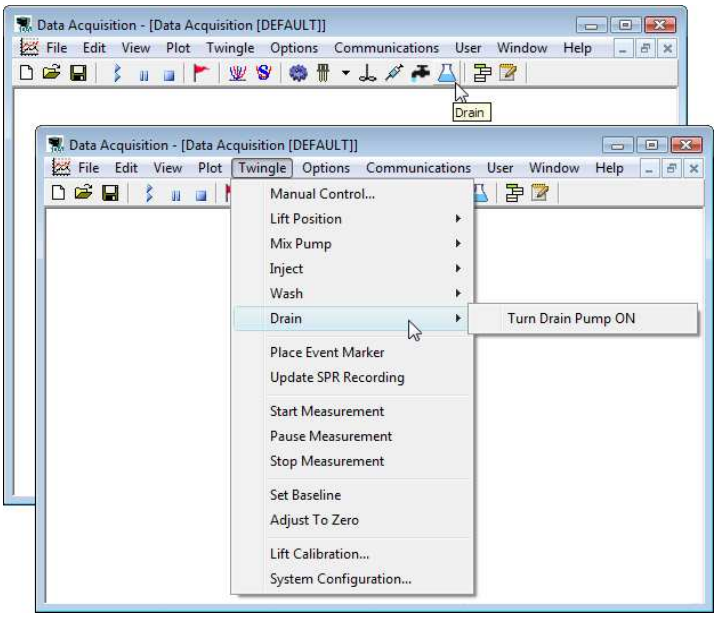


Figure 5.19 – Direct access to start and stop the drain pump.

5.7.6 – Place Event Marker.

To mark a position on the data line during a measurement select the button and chose the position on the data curve. This button will mark an event (for example the association and dissociation steps) in the measurement window and describe this event in the event log window. Events indicated by a marker will be recorded in the event log.

5.7.7 – Update SPR recording.

Records a SPR dip during the measurement. As shown in figure 4.20, there are three different possibilities to have an update SPR recorded. A SPR update is necessary to check if there is a gold disk quality problem or an air bubble present on the gold disk during an experiment.
All updates are automatically stored in the event log. The plots are visualized in the SPR 1 plot and SPR 2 (Twingle only) plot (Figure 5.1). Every update can be shown or hidden with a right mouse click. In Figure 5.21, all updates are selected, but they can also be (de-)selected individually. The maximum number is 20 updates.

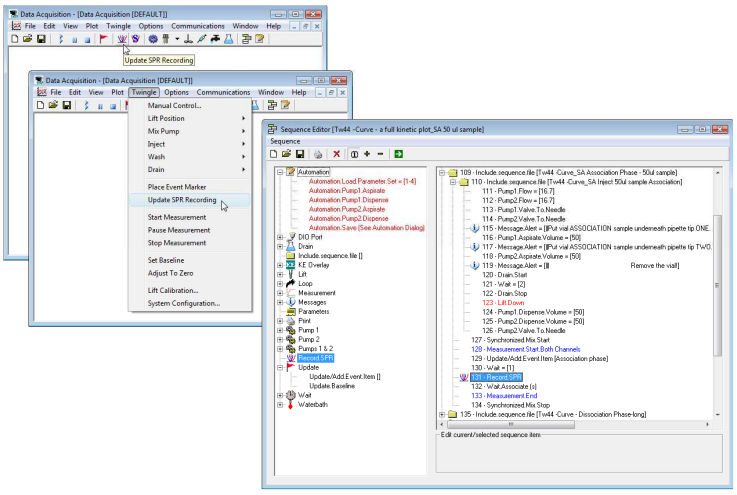


Figure 5.20 – Update SPR Recording.

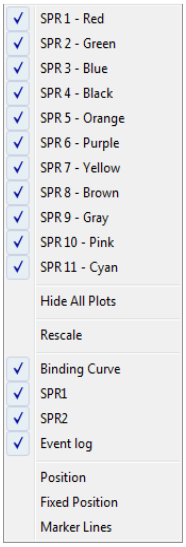


Figure 5.21 – Right mouse click on SPR plot 1 or on SPR plot 2.

5.7.8 – Start measurement.

Start measurement will plot SPR data in the data acquisition plot, angle vs. time. Each interval time, the angle at which the SPR minimum occurs, is determined and plotted in the DA window.

5.7.9 – Pause measurement.

Pauses plotting SPR minimum in de Binding Curve plot, although the time is still proceeding on the background. A restart will draw a straight line between the last plotted SPR data point and the first new SPR data point from which the measurement will proceed.

5.7.10 – Stop measurement.

Stops recording SPR. After measurement start, the measurement continues with the last recorded data point.

5.7.11 – Set Baseline.

A set of data points which shows a horizontal line in the binding curve can be marked as baseline with this option. All following events are recorded with a relative response to this baseline, the relative response values are saved in the event log.

5.7.12 – Adjust to zero.

Allows user to adjust the measured SPR angle to zero. The raw data, recorded after adjust to zero, is modified with an offset, in contrast to the previous option. The “adjust to zero” option can be used to adjust both channels to zero.

5.7.13 – Lift Calibration.

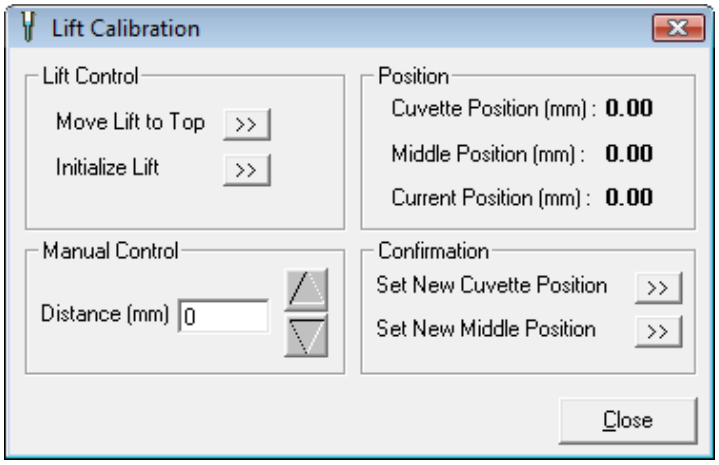


Figure 5.22 – Lift calibration window.

The “lift calibration” calibrates the pipette position. The cuvette position for the pipette is normally calibrated to 1 mm above the gold disk, the middle position is calibrated with the pipette just about 2 mm inside the cuvette.
Procedure (specified distances depend on the size of the used pipette tips):

- Initialize lift.
- Enter a distance (max. 60 mm). Start with e.g. 54 mm and fine tune with 1 mm steps.
- Set new cuvette position.
- Move lift to top
- Enter distance of +/- 42 mm
- Set new middle position
- Move lift to top
- Close.

5.7.14 – System Parameters.

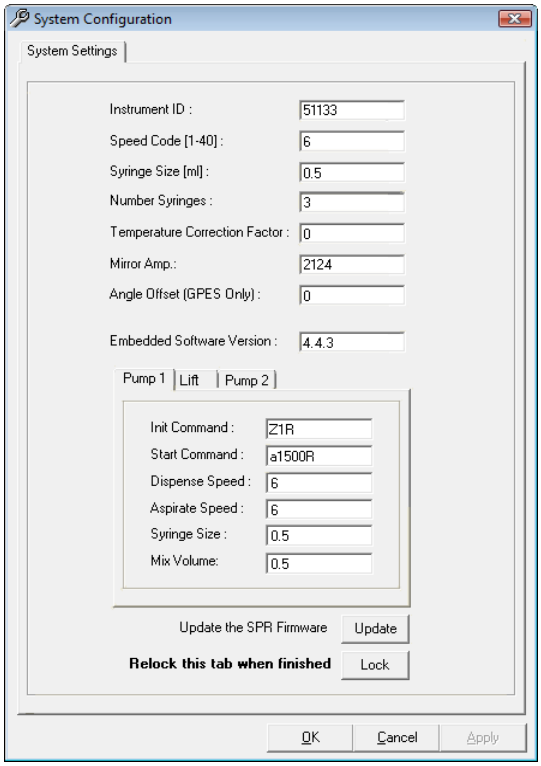


Figure 5.23 – System settings.
Tab sheet “pump2” for Twingle only.

System settings
All hardware setup settings are defined in the System Settings. These numbers should not be changed! One important number to read is the “Mirror Amp.” which refers to a specific instrument unique dynamic scanning

angle range. So, the number 2124 defines the dynamic scanning range of 4248 millidegrees. The number 2124 needs to be written in the NOVA settings when performing ESPR measurements or should be checked in the C:Windows\ESPR.ini file for the GPES software.

5.8 – Options menu.

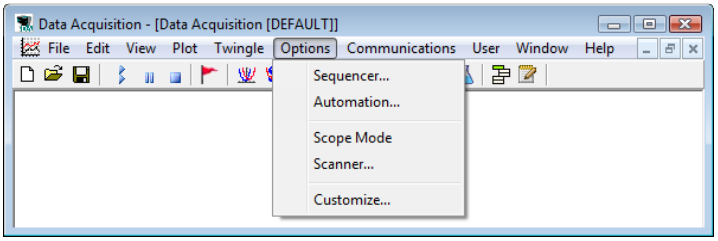


Figure 5.24 – Options menu.

5.8.1 – Sequencer.

The SPR sequence editor is used to automate measurements. See chapter 5.

5.8.2 – Automation.

Opens the Automation window. Here experimental parameters, incubation times and storage file name can be addressed. See chapter 6.

5.8.3 – Scope mode.

Updates the SPR dip every 0.5 seconds. Useful for manually adjusting the optical path.

5.8.4 – Scanner.

Stops and starts the scanner. Only used for service items.

5.8.5 – Customize.

Opens a window to specify software settings.
General settings tab page

- Include filename in plot when printing:
  Check this box to put the filename on the printouts.
- Alert before executing a changed sequence:

Before the measurement will be executed, an alert is shown to confirm the sequence change before starting the measurement.

- Clear experimental title at new measurement:
  Clear the experiment title before each start of a new measurement.
- Try to connect SPR at start-up:
  If the instrument power is ON, the software will automatically connect to the instrument at start-up.
- Initial temperature of water bath [°C]:
  This command field set the water temperature in the water bath. The waterbath must be switched on before starting the Twingle software.

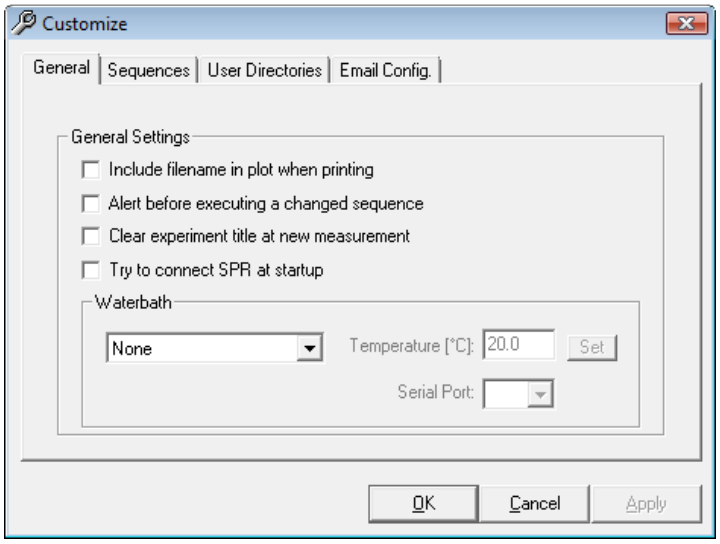


Figure 5.25 – Customize – General tab page.

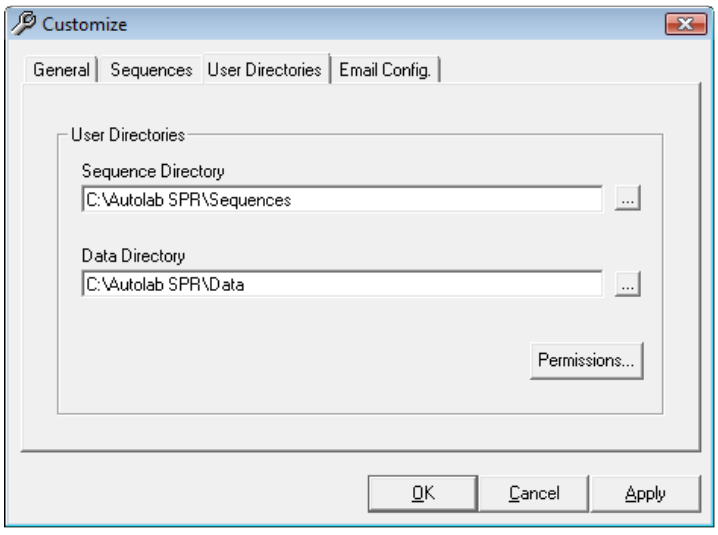


Figure 5.26 – Customize - User directories tab page.

Sequences

See paragraph §5.7.3, figure 5.15.

User directories tab page (See Figure 5.26)

The user can set default paths for a sequence and a data directory.

Email configuration tab page.

Configure for the email address of the user to allow the email message command in the sequencer to send an email message.

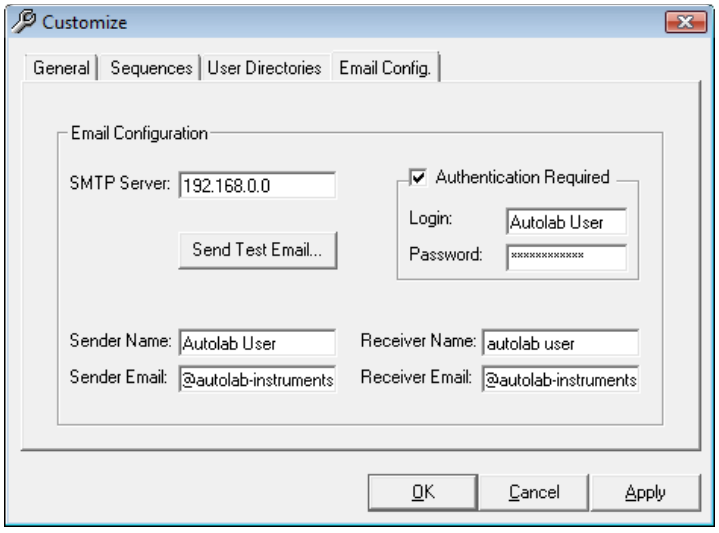


Figure 5.27 – Customize – Email configuration tab page.

5.9 – Communications menu.

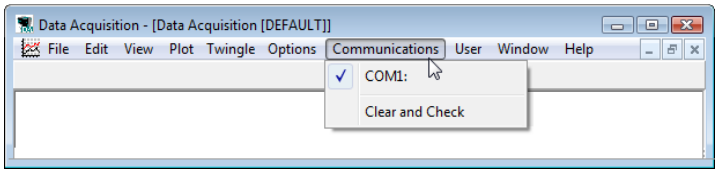


Figure 5.28 – Communications.

Serial port

The software will automatically check for COM1 up to COM5. Check the correct COM port to start connection with the 'clear and check' option. The other COM port can be used for controlling a waterbath.

Clear and check Re-establish the communication between the system and the software.

In cases where it is necessary to restart the software, the clear and check option can be used to reconnect the instrument with the computer.

5.10 – User menu (optional).

A separate manual specific for the Good Laboratory Practice features is written. Here only a few screen shots are shown as examples.

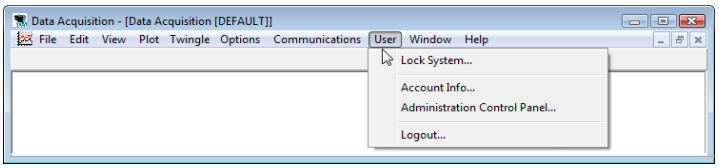

Data Acquisition - [Data Acquisition [DEFAULT]]
File Edit View Plot Twingle Options Communications User Window Help
Lock System...
Account Info...
Administration Control Panel...
Logout...

Figure 5.29 – User.

Administration Control Panel

Add or delete User accounts or groups, regulates the user access rules, shows the user configuration settings. Keeps track of all actions in the Audit trail.

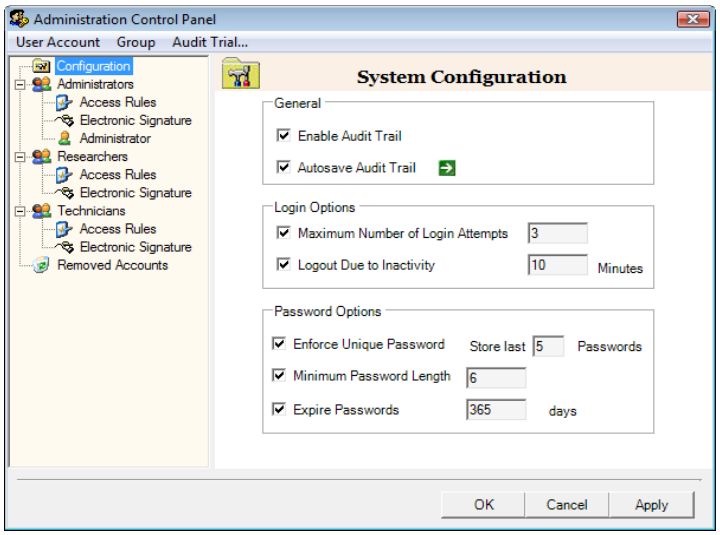


Figure 5.30 – The Administration Control Panel.

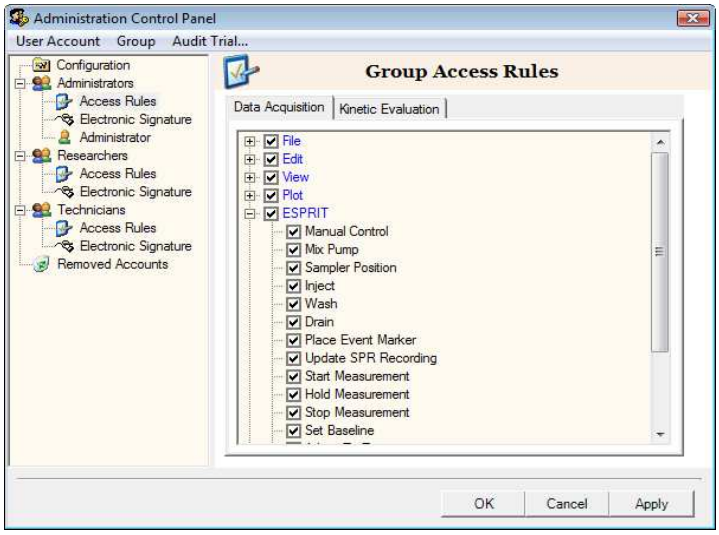


Figure 5.31 – The Administration Control Panel. The access rules per group can be limited. Every menu bar item with its commands can be (de-) selected.

5.11 – Window menu.

Cascade	Places several overlapping windows in a cascade.
Tile	All binding plot curves windows are tiled within the data acquisition window next to each other.
Arrange icons	Arrange data acquisition icons.
Close all	Close all data presentation windows, the data acquisition window cannot be closed.

5.12 – Help menu.

Graph action help	Help on graph commands.
About SPR software	Software version information.

5.13 – Event Log.

The Event Log can be selected in the menu VIEW. As the name indicates, all events during an experiment are stored in this log.
These events are:

- Update SPR.
  See examples in Figure 4.32 with the lines; 'Update SPR-[Blue]', Green, Cyan, Yellow, Black. Each event is recorded with time, angle, temperature, relative response and a text line for comments.

- Set Baseline (menu Twingle).
  See Figure 4.33 with the lines; 'New baseline value, relative response is set to zero m°'. Notice the column 'angle [m°]' and the column 'Rel(ative) Response [m°], before and after the 'New baseline' action line. Afterwards the angle value in the Rel Response column is set to zero to be able to read the angle shift during the experiment.

- Place marker and 'update / add event.item [ ]'.
  Both actions are used to create an event line in the event log to indicate what happened during the measurement. The Update/add event item is a command line in the sequence editor (see chapter 6). Use "Place marker" to get a number in the data acquisition plot and in the event log. In the event log a remark can be added to this marker.
  If the Update/add event item is used as a command in a sequence, then the remark is already specified between the brackets in the sequence. See for example the event text lines like '1:[association]' and '2:[association]' in Figure 4.32 and Figure 4.33.

Remarks:
- The event log remarks can be edited with a double click on the line.
- Deleting event log lines is also possible. If a marker is deleted and a new marker is put onto the DA plot, the event log will continue to increment the number. If the position of the new marker is coincidently the same position as the deleted marker, the old marker number will be used.
- A SPR plot can handle up to twelve updates of recorded dips.

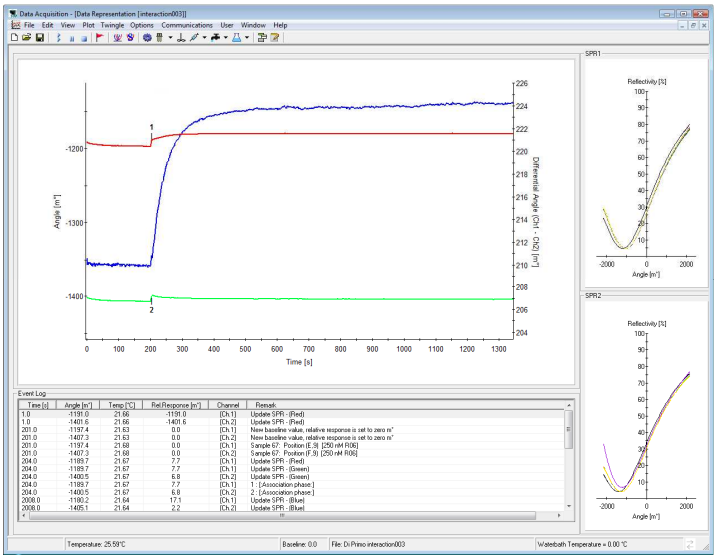


Figure 5.32 – An example of a kinetic experiment, with event log data. The upper line is channel 1, the lower line is channel 2 data. The middle curve is the differential angle curve between channel 1 and 2.

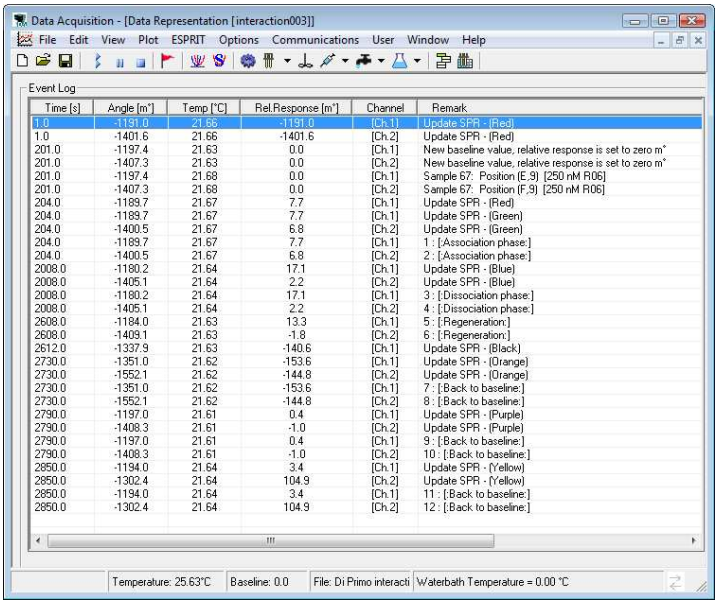

Data Acquisition - [Data Representation [interaction003]]
File Edit View Plot ESPRIT Options Communications User Window Help
Event Log
Time [s] Angle [m°] Temp [°C] Rel.Response [m°] Channel Remark
1.0 -1191.0 21.66 -1191.0 [Ch.1] Update SPR - (Red)
1.0 -1401.6 21.66 -1401.6 [Ch.2] Update SPR - (Red)
201.0 -1197.4 21.63 0.0 [Ch.1] New baseline value, relative response is set to zero m°
201.0 -1407.3 21.63 0.0 [Ch.2] New baseline value, relative response is set to zero m°
201.0 -1197.4 21.68 0.0 [Ch.1] Sample 67: Position (E,9) [250 nM R06]
201.0 -1407.3 21.68 0.0 [Ch.2] Sample 67: Position (F,9) [250 nM R06]
204.0 -1189.7 21.67 7.7 [Ch.1] Update SPR - (Red)
204.0 -1189.7 21.67 7.7 [Ch.1] Update SPR - (Green)
204.0 -1400.5 21.67 6.8 [Ch.2] Update SPR - (Green)
204.0 -1189.7 21.67 7.7 [Ch.1] 1 : [:Association phase:]
204.0 -1400.5 21.67 6.8 [Ch.2] 2 : [:Association phase:]
2008.0 -1180.2 21.64 17.1 [Ch.1] Update SPR - (Blue)
2008.0 -1405.1 21.64 2.2 [Ch.2] Update SPR - (Blue)
2008.0 -1180.2 21.64 17.1 [Ch.1] 3 : [:Dissociation phase:]
2008.0 -1405.1 21.64 2.2 [Ch.2] 4 : [:Dissociation phase:]
2608.0 -1184.0 21.63 13.3 [Ch.1] 5 : [:Regeneration:]
2608.0 -1409.1 21.63 -1.8 [Ch.2] 6 : [:Regeneration:]
2612.0 -1337.9 21.63 -140.6 [Ch.1] Update SPR - (Black)
2730.0 -1351.0 21.62 -153.6 [Ch.1] Update SPR - (Orange)
2730.0 -1552.1 21.62 -144.8 [Ch.2] Update SPR - (Orange)
2730.0 -1351.0 21.62 -153.6 [Ch.1] 7 : [:Back to baseline:]
2730.0 -1552.1 21.62 -144.8 [Ch.2] 8 : [:Back to baseline:]
2790.0 -1197.0 21.61 0.4 [Ch.1] Update SPR - (Purple)
2790.0 -1408.3 21.61 -1.0 [Ch.2] Update SPR - (Purple)
2790.0 -1197.0 21.61 0.4 [Ch.1] 9 : [:Back to baseline:]
2790.0 -1408.3 21.61 -1.0 [Ch.2] 10 : [:Back to baseline:]
2850.0 -1194.0 21.64 3.4 [Ch.1] Update SPR - (Yellow)
2850.0 -1302.4 21.64 104.9 [Ch.2] Update SPR - (Yellow)
2850.0 -1194.0 21.64 3.4 [Ch.1] 11 : [:Back to baseline:]
2850.0 -1302.4 21.64 104.9 [Ch.2] 12 : [:Back to baseline:]
Temperature: 25.63°C
Baseline: 0.0
File: Di Primo interacti
Waterbath Temperature = 0.00 °C

Figure 5.33 – A zoom-in on the event log from Fig. 5.32. A double click on a remark will result in an editable line below the event log window.

Chapter 6

6 – Sequencer.

6.1 – Index.

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6.2 – Introduction.

The sequence editor is a powerful tool to automate experiments. Sequences can be used to describe experiment parameters (i.e. flow speed, mix volume, sample volume, etc.), sample positions, measurement times and liquid handling. In general, sequences are used for automatic or semi-automatic control of an experiment.

6.3 – Sequence editor window.

Select from the toolbar or use menu – Options – Sequencer… to open the Sequence Editor window.

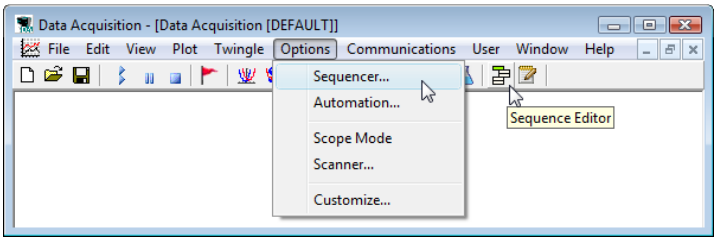


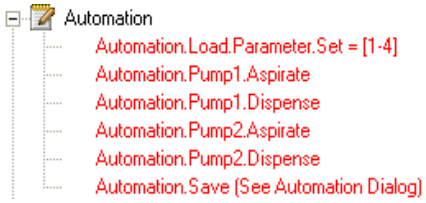
Figure 6.1 – Two ways to activate the Sequencer, via the Menu-Options or the Toolbar button.

The sequence editor window contains a list of simple commands in the left window and a list of commands in sequence to the right window. The list of commands on the right forms a sequence.

By using the double click, the selected command from the “left” will be added at the bottom of the assembled sequence. The drag and drop functionality allows inserting a specific command at a selected position on the “right” in the assembled sequence. A user-defined sequence can be stored or retrieved from disk, it will have the extension *.seq.

A sequence can execute other (include) sequences, this makes it possible to develop a standard set of sequences for general purposes. These subroutine sequences are called “include-sequences”. During installation of the software a list of sequences and include-sequences are stored in the Autolab SPR software directory.

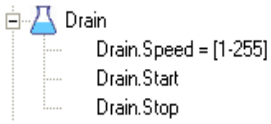
All sequence commands and their functions are listed below.



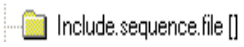
Pump2 commands are available in Twingle only.

- Loads the parameters settings determined in the automation control window 'parameters tab'.
- Aspirate the sample volume, filled out in the 'volume tab', in pump 1 for channel 1.
- Dispense the sample volume, filled out in the 'volume tab', in pump 1 for channel 1.
- Aspirate the sample volume, filled out in the 'volume tab', in pump2 for channel 2.
- Aspirate the sample volume, filled out in the 'volume tab', in pump 2 for channel 2.
- Saves data. Fill out a file name in the Sampler Window.

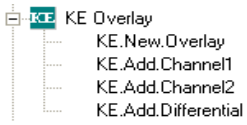
- Sends out a DIO port trigger to the PGSTAT.
- Receives a DIO port trigger from the PGSTAT.



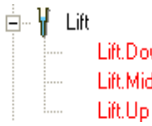
- Defines the drain, left peristaltic pump (pump 3), 40rpm speed in 255 steps.
- Drains the cuvette.
- Stops draining the cuvette.



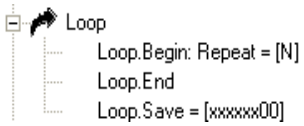
Inserts sequence file (*.seq) in opened sequence.



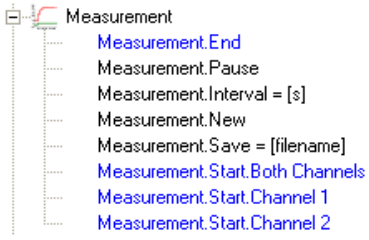
- Opens KE software and creates a new overlay.
- Add the channel 1 data to the KE overlay
- Add the channel 2 data to the KE overlay
- Add the differential data to the KE overlay



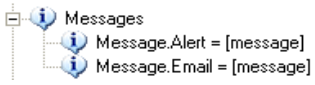
Sends the needles/pipette tips to the;
-Inject positon, 1mm above the gold
- Just within the cuvette position
- Home position



- Starts loop in sequence, N defines number of cycles (linked to Loop.End command).
- Stops loop (linked to Loop.Begin).
- Saves the loop file by name + counter (e.g. protein001)

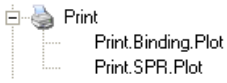


- Stops measurement
- Holds measurement plot, but the time is recorded
- Defines measurement interval time in seconds (0.1s – 300s)
- Clears the DA plot window and sets time to zero
- Saves measurement.
- Starts measurement in channel 1 and channel 2
- Starts measurement in channel 1
- Starts measurement in channel 2

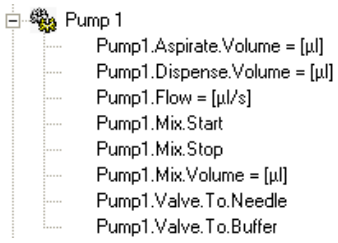


- Shows message box with [message] and postpones sequence until message is confirmed by “Continue” or “Abort” button
- Sends out an email, which is configured in Options_ Customize_ email.

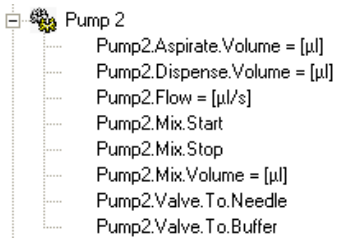
Opens a window with all measurement parameters



- Prints a hardcopy of the binding plot.
- Prints a hardcopy of the SPR signal.



- Defines aspirate volume in µl for channel 1.
- Defines dispense volume in µl for channel 1.
- Defines pump 1 flow in µl/s for channel 1.
- Starts mixing pump 1(advice: always in Lift.Down position) .
- Stops mixing pump 1.
- Defines mix volume in µl for channel 1.
- Switches pump valve position to needle/pipette tip for pump 1.
- Switches pump valve position to buffer for pump 1



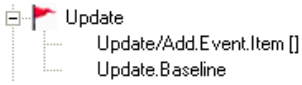
Twingle only

- Defines aspirate volume in µl for channel 2.
- Defines dispense volume in µl for channel 2.
- Defines pump flow in µl/s for channel 2.
- Starts mixing pump 2 (advice: always in Lift.Down position).
- Stops mixing pump 2.
- Defines pump 2 mix volume in µl for channel 2.
- Switches pump valve position to needle/pipette tip for pump 2.
- Switches pump valve position to buffer for pump 2

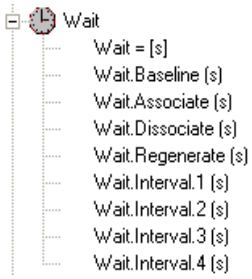
Twingle only

- Defines pump 1 & 2 flow in µl/s for channel 1 and 2
- Starts mixing pump 1 & 2
- Stops mixing pump 1 & 2
- Defines pump 1 & 2 mix volume in µl for channel 1 and 2

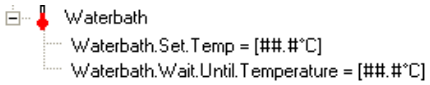
Records an SPR plot for channel 1 and 2.



- Adds event with text to event recorder.
- Set Relative Response to zero.



- Wait period in seconds.
- Baseline wait period. Defined in the Automation Window.
- Associate wait period. I Defined in the Automation Window.
- Dissociate wait period. Defined in the Automation Window.
- Regenerate wait period. Defined in the Automation Window.
- Wait periods. Defined in the Automation Window.
- Wait periods. Defined in the Automation Window.
- Wait periods. Defined in the Automation Window.
- Wait periods. Defined in the Automation Window.



- Set temperature of the waterbath, (Julabo or Lauda)
- Set temperature of the waterbath and wait until the temperature is reached before continuing with the next sequence step.

6.4 – Software Sequence editor.

The Sequence Editor toolbar, see Figure 5.3, contains the shortcuts to the menu items: new sequence, open sequence, save sequence, print, numbering and execute respectively.

The functions for the menu and toolbar items will be explained in the next section.

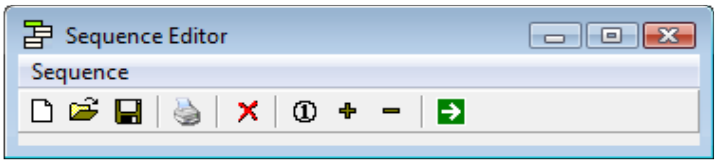


Figure 6.3 – The menu bar and tool bar buttons.

6.4.1 – The sequence editor menu and toolbar.

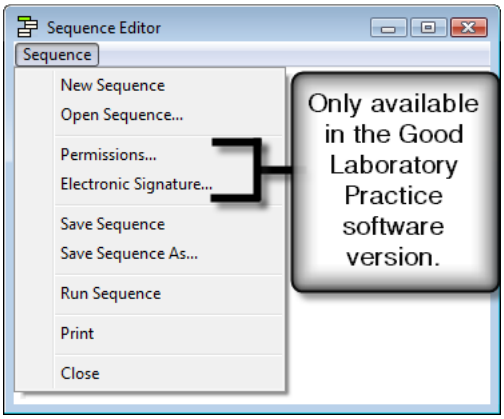


Figure 6.4 – The sequence menu.

New Sequence Clears the sequence editor window.

	Open Sequence	Opens a sequence folder determined in the customize – user directories tab (Figure 5.33, p.122).
	Save Sequence	Saves the sequence.
	Save Sequence As	Opens a 'save as' window to save the sequence as SEQ file, with a user created filename and directory.
	Print	Prints the complete command structure of the opened sequence.
	Delete	Delete highlighted command line.
	Numbering	Shows or hides the numbering of the command lines.
	Expand	Shows all sequence commands lines
	Collapse	Shows only the main sequence command lines.
	Run Sequence	Executes the current sequence.
	Close	Closes the sequence editor window.

6.5 – Set-up of sequence files.

6.5.1 – Include-sequence.

All of the basic liquid handling commands are stored in so called 'include-sequence' files. This reduces the number of lines in a main sequence and gives a better overview on the executed sequence. An include-sequence executed as a main sequence, may cause problems. Check the sequence with a buffer to verify if it can be used as a main sequence.
A sequence should be considered as the folder structure on the hard drive of the PC. An "include sequence" can be described as the function 'folder' in the 'directory'. The "include sequence" also has many commands, that can be read when clicking on the 'plus' box (like in widows explorer). A list of include sequences can be found under C:\Autolab SPR\Sequencer\Include.

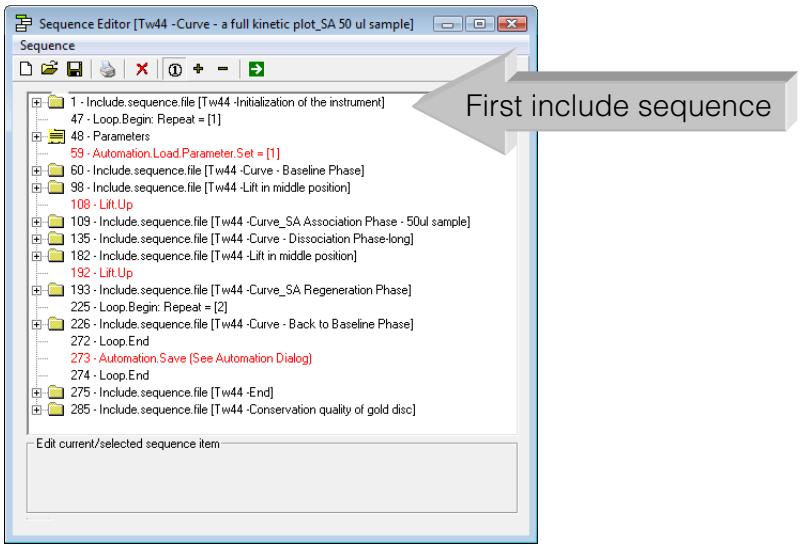

Sequence Editor [Tw44 -Curve - a full kinetic plot_SA 50 ul sample]
Sequence
1 - Include.sequence.file [Tw44 -Initialization of the instrument]
47 - Loop.Begin: Repeat = [1]
48 - Parameters
59 - Automation.Load.Parameter.Set = [1]
60 - Include.sequence.file [Tw44 -Curve - Baseline Phase]
98 - Include.sequence.file [Tw44 -Lift in middle position]
108 - Lift.Up
109 - Include.sequence.file [Tw44 -Curve_SA Association Phase - 50ul sample]
135 - Include.sequence.file [Tw44 -Curve - Dissociation Phase-long]
182 - Include.sequence.file [Tw44 -Lift in middle position]
192 - Lift.Up
193 - Include.sequence.file [Tw44 -Curve_SA Regeneration Phase]
225 - Loop.Begin: Repeat = [2]
226 - Include.sequence.file [Tw44 -Curve - Back to Baseline Phase]
272 - Loop.End
273 - Automation.Save (See Automation Dialog)
274 - Loop.End
275 - Include.sequence.file [Tw44 -End]
285 - Include.sequence.file [Tw44 -Conservation quality of gold disc]
Edit current/selected sequence item
First include sequence

Figure 6.5 – Example of a sequence with include-sequences. An include sequence function resembles a folder in window explorer.

6.5.2 – Safety lines.

To prevent flooding of the cuvette and to prevent stock solution contamination, some safety measures are necessary. After the Pump1.Mix.Stop, Pump2.Mix.Stop (Twingle only) or Synchronized.Mix.Stop (Twingle only) command, the syringe pump will automatically go to its initialization position. The initial plunger position is half way the syringe (which is the 250 µl point). Another situation, at which the pumps will go to their initial position, is after finishing a sequence.
To prevent mistakes, use the Synchronized.Mix.Stop command as a start of an include sequence. From that point on it is clear what the limits are in aspirate and dispense volume:

- Pump1.flow=[227.3] µl/s
- Pump2.flow=[227.3] µl/s (Twingle only)
- Pump1.Valve.To.buffer
- Pump2.Valve.To.buffer (Twingle only)
- Synchronized.Mix.Stop (Twingle only)

(Pump.Mix.Stop (Springle only))

The changes in the syringe position will not affect the buffer and/or sample level in the needle and/or cuvette.

6.5.3 – Wait command.

A Wait command used as an incubation time for a measurement is positioned in between a Synchronized.Mix.Start with Measurement.Start.Both.Channels and a Measurement.End with Synchronized.Mix.Stop command. In case of a

xxxxx.Mix.Start before the Wait command also a xxxxx.Mix.Stop after the Wait command is necessary.
If a wait command is active without a Measurement.Start command active, the temperature registration in the software is not updated. In this case the temperature is not updated in the software.

6.5.4 – Save data.

There are three different commands to automatically save data while executing a sequence.

6.5.4.1 – Loop.Save= [xxxxxx00].

Data measured during the sequential execution of a loop command, will be stored under the same name with an increasing serial number.
A proper filename has to be specified before starting the sequence.

Within a sequence:

- Loop.Begin:Repeat = [N] N defines number of cycles,
- Loop. Save = (xxxxxx00) like “sample00” or “3April00”
- Loop.end

The part of the sequence between the Loop.Begin:Repeat and Loop.End will be repeated N times.

6.5.4.2 – Measurement.Save = [filename].

At the end of an experiment the data are saved using the specified filename. This command is useful at the end of a sequence to save one defined experiment.

6.5.4.3 – Automation.Save [see automation window].

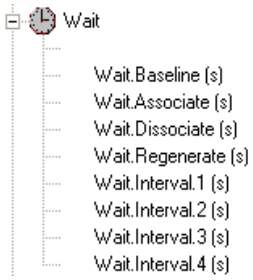
This is a command for a sequence that uses input from the Autosampler window. Specify a filename in the Automation window. The data are automatically saved using this filename. All samples will have the same filename with an increasing serial numbers at the end.

6.5.5 – Commands with variables.

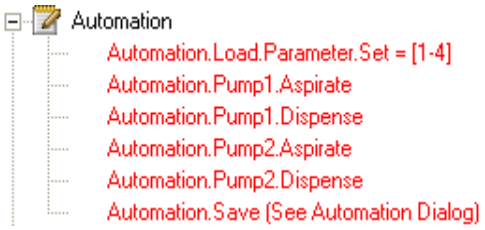
Command	Valid entry
Drain.Speed = [1-255]	between 1 and 255 rpm (≤120 ul/s)
Loop.Begin: Repeat = [N]	no limitation
Measurement.Interval = [s]	between 0.1 and 300
Waterbath set temp = ##.# [°C]	between 10.0 – 70.0
Waterbath set and wait = ##.# [°C]	between 10.0 – 70.0
Pump (1 or 2).Aspirates.Volume = [µl]	between 1 and 500
Pump (1 or 2).Dispense.Volmue = [µl]	between 1 and 500
Pump (1 or 2).Flow = [µl/s]	between 227.3 and 0.8, in 31 steps
Synchronized.Mix. Flow = [µl/s]	between 227.3 and 0.8, in 31 steps
Pump (1 or 2).Mix.Volume = [µl]	between 1 and 100
Synchronized.Mix.Volume = [µl]	between 1 and 100

6.5.6 – Commands for Semi-Automatic sequences.

Semi-Automatic sequences are generally sequences used in combination with the Automation window. A number of commands are linked to the Automation control window. Those commands can not be specified in the sequence editor window, they are linked to a specific figure filled out in the Automation window. (menu –Options; Automation). For example, the 'Wait' table below is linked with the tab sheet 'time' in the Automation control window. See also table 6.1



- Baseline wait period.
  Input in the Automation Window 'time tab'.
- Associate wait period.
  Input in the Automation Window 'time tab'.
- Dissociate wait period.
  Input in the Automation Window 'time tab'.
- Regenerate wait period.
  Input in the Automation Window 'time tab'.
- Interval.1 (Automation 'time tab').
- Interval.2 (Automation 'time tab').
- Interval.3 (Automation 'time tab').
- Interval.4 (Automation 'time tab').



Pump 2 commands are available in Twingle only.

- Loads the parameters settings determined in the automation control window 'parameters tab'.
- Aspirate the sample volume, filled out in the 'volume tab', in pump 1 for channel 1.
- Dispense the sample volume, filled out in the 'volume tab', in pump 1 for channel 1.
- Aspirate the sample volume, filled out in the 'volume tab', in pump2 for channel 2.
- Aspirate the sample volume, filled out in the 'volume tab', in pump 2 for channel 2.
- Saves data. Input file name in the Sampler Window.

6.5.6.1 – The main automatic kinetic sequence with all of its included sequences.

The sequences mentioned below are for double channel synchronized. The basic of all the sequences is the same, just small changes serve different measurement requirements. (see fig. 6.7)

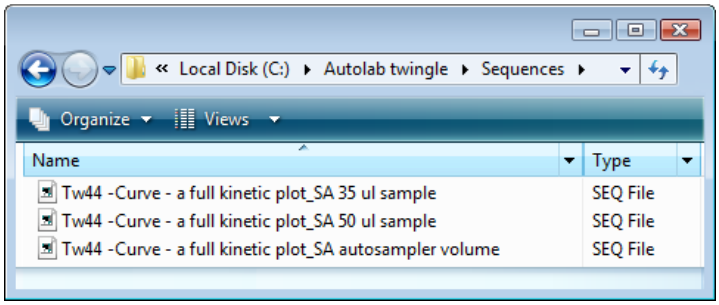

« Local Disk (C:) ▸ Autolab twingle ▸ Sequences ▸
Organize
Views
Name
Type
Tw44 -Curve - a full kinetic plot_SA 35 ul sample
SEQ File
Tw44 -Curve - a full kinetic plot_SA 50 ul sample
SEQ File
Tw44 -Curve - a full kinetic plot_SA autosampler volume
SEQ File

Figure 6.6 A. – Twingle List of kinetic experiment sequences.

For the TWINGLE the Tw44- Curve - a full kinetic plot_SA 50µl sample.seq sequence is the main sequence from which all other sequences are generated.

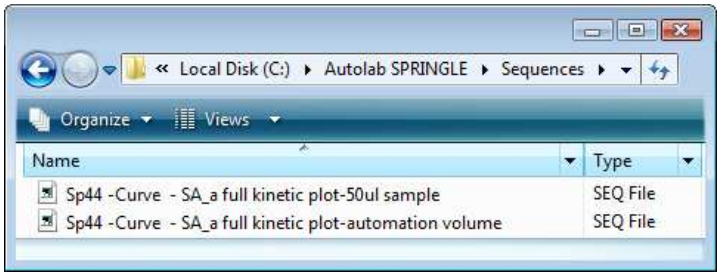


Figure 6.6 A. – Springle list of kinetic experiment sequences.

For the SPRINGLE the Sp44- Curve –SA_a full kinetic plot_SA 50µl sample.seq sequence is the main sequence from which the other sequence is generated.

For the explanation of the automation the Twingle has been used as an example. The Springle has the same setup.
What and where are the changes to make different sequences is shown below. Commands in lines 108,192 shows the Lift up position which indicates that in the next folder (include sequence) the aspiration of a sample (lines 116, 118) needs to come from a vial underneath the pipette tip and lines 124,125 are dispensing the sample into the cuvette. It is therefore very easy to adjust the sequence to a new volume. Even better, the right sequence has a link with the automation control window where the volume can be filled out (see fig. 6.7).

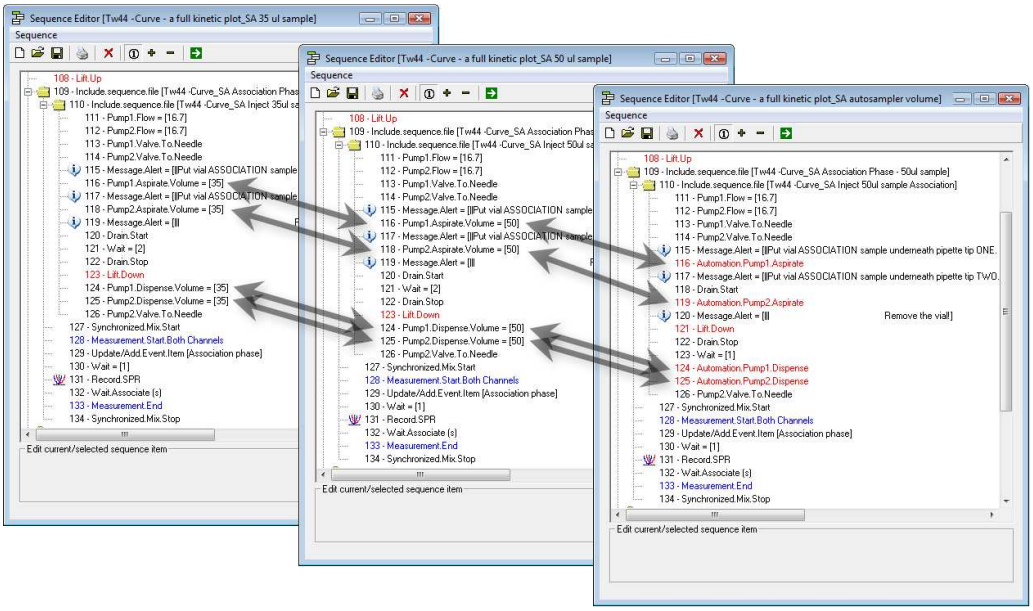

Sequence Editor [Tw44 -Curve - a full kinetic plot_SA 35 ul sample]
Sequence
Sequence Editor [Tw44 -Curve - a full kinetic plot_SA 50 ul sample]
Sequence
Sequence Editor [Tw44 -Curve - a full kinetic plot_SA autosampler volume]
Sequence
Edit current/selected sequence item

Figure 6.7 – The difference in sample volume.

Tw44 -Curve - a full kinetic plot.seq	Main sequence
Tw44 -Curve - Baseline phase.seq	First level of include-sequences
Tw44 -Curve - Association phase.seq	
Tw44 -Curve - Dissociation phase.seq	
Tw44 -Curve - Regeneration phase.seq	
Tw44 -Curve - Back to Baseline phase.seq	
Tw44 -Curve - Inject 50 µl Buffer Baseline.seq	Second level of include-sequences sets the sample volume. Sample volume commands are the pump aspirate volume and command pump dispense volume
Tw44 –Curve_SA_Inject 50 µl sample Association.seq	
Tw44 -Curve - Inject 50 µl Buffer Dissociation.seq	
Tw44 –Curve_SA_Inject 50 µl sample Regeneration.seq	
Tw44 -Curve - Inject 50 µl Buffer Back to Baseline.seq	

Table 6.1; The order of incubation times per sequence.

Include sequence / Sequence: *.SEQ	Initialization	TW44 -Curve Baseline Phase	Tw44 -Curve Association phase	Tw44 -Curve Dissociation phase	Tw44 -Curve Regeneration phase	Tw44 -Curve Back to Baseline phase	end	Tw44 – Conservation QA gold
		time	time	time	time	time		time
Tw44 -Curve - a full kinetic plot_SA 35 ul sample			**35 ul sample**					
		Wait.Baseline [s]	Wait.association [s]	Wait.dissociation [s]	Wait.regeneration [s]	Wait.interval.1[s]		Wait [s]
Tw44 -Curve - a full kinetic plot_SA 50 ul sample			**50 ul sample**					
		Wait.baseline [s]	Wait.association [s]	Wait.dissociation [s]	Wait.regeneration [s]	Wait.interval.1[s]		Wait [s]
Tw44 -Curve - a full kinetic plot_SA adjustable volume		**Flexible sample volume**	**Flexible sample volume**	**Flexible sample volume**	**Flexible sample volume**	**Flexible sample volume**		**Flexible sample volume**
		Wait.baseline [s]	Wait.association [s]	Wait.dissociation [s]	Wait.regeneration [s]	Wait.interval.1[s]		Wait [s]

To be able to fill out the “time” tab page in the automation control window, the knowledge of the sequence to be used is necessary. This knowledge can be gained by opening the sequence in the sequence editor window and reading the sequence. The table below shows sequences and their order of time commands used in measurements. The SPRINGLE sequences use the same set of incubation time commands!

6.5.6.2 – The interaction plot sequence.

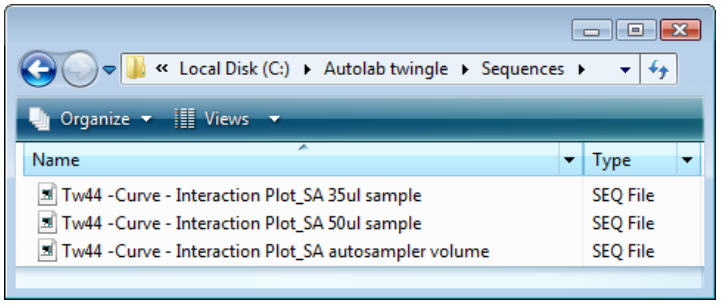


Figure 6.8 A. – Twingle list of interaction experiment sequences.

The interaction plot sequence is an almost exact copy of the sequence called “Tw44 -Curve- a full kinetic plot.seq”. The only difference is the absence of the dissociation phase. This sequence can be used if the dissociation constant is not of interest, like for affinity constant or for qualitative results.

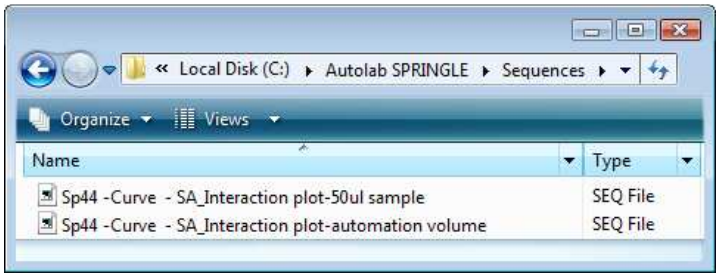


Figure 6.8 B.– Springle list of interaction experiment sequences.

For the SPRINGLE the Sp44- Curve –SA_Interaction plot-50µl sample.seq sequence is the main sequence from which the other sequence is generated.

For the explanation of the automation the Twingle has been used as an example. The Springle has the same setup.
To be able to fill out the “time” tab page in the automation control window, the knowledge of the sequence to be used is necessary. This knowledge can be gained by opening the sequence in the sequence editor window and reading the sequence. The table below shows sequences and their order of time commands used in measurements.

TW44 –Curve - Interaction plot - 50ul sample.seq	Main sequence.
Tw44 -Curve - Baseline phase.seq	Include-sequences inside the main sequence.
Tw44 -Curve - Association phase.seq	
Tw44 -Curve - Regeneration phase.seq	
Tw44 -Curve - Back to Baseline phase.seq	
Tw44 -Curve - Inject 50 µl Buffer Baseline.seq	Second level of include-sequences.
Tw44 –Curve_SA_Inject 50 µl sample Association.seq	
Tw44 –Curve_SA_Inject 50 µl sample Regeneration.seq	
Tw44 -Curve - Inject 50 µl Buffer Back to Baseline.seq	

Table 6.2; The order of incubation times per sequence.

Include sequence / Sequence: *.SEQ	Initialization	Tw44 -Curve Baseline Phase	Tw44 -Curve Association phase	Tw44 -Curve Regeneration phase	Tw44 -Curve Back to Baseline phase	end	Tw44 – Conservation QA gold
		time	time	time	time		time
Tw44 -Curve - Interaction plot_SA 35 ul sample			**35 ul sample**				
		Wait.Baseline [s]	Wait.association [s]	Wait.regeneration [s]	Wait.interval.1[s]		Wait [s]
Tw44 -Curve - Interaction plot_SA 50 ul sample			**50 ul sample**				
		Wait.baseline [s]	Wait.association [s]	Wait.regeneration [s]	Wait.interval.1[s]		Wait [s]
Tw44 -Curve - Interaction plot_SA adjustable volume		**Flexible sample volume**	**Flexible sample volume**	**Flexible sample volume**	**Flexible sample volume**		**Flexible sample volume**
		Wait.baseline [s]	Wait.association [s]	Wait.regeneration [s]	Wait.interval.1[s]		Wait [s]

6.5.6.3 – The inject sequence

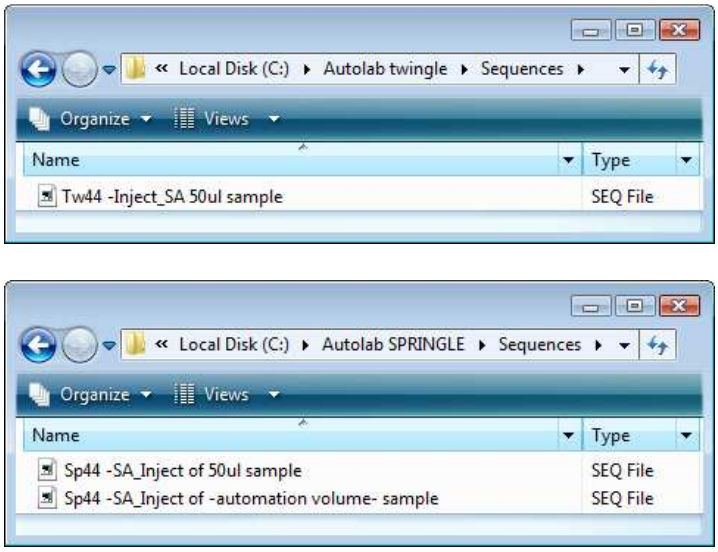


Figure 6.9 – The inject sequence.

There are two ways to start these sequences, by using the sequence editor window and by using the inject button in the toolbar. See paragraph 5.7.3 inject. It will inject one sample from one position and start the measurement with an incubation time 'Wait [s]' (=60). The inject sequence has the "Wait [s]" command in its sequence. So, the incubation time has to be changed in the sequence itself. To change the time (or any other item between brackets), double click on the command and in the edit part of the window the command with its value to be changed, is shown. Whenever the "Wait[s] has been changed in one of the other "Wait.xxxx [s]" commands, the inject sequence can also be executed via the Automation window.
The toolbar button inject (icon) will activate the Customize - Inject window. In this window a sequence can be chosen and the measurement time in the inject sequence can be specified. The incubation time wait [s] as specified in the sequence will be overruled by this specified measurement time.

6.5.6.4 – The stabilization sequences.

These types of sequences have all the incubation command 'wait [s]' to be filled out in the sequence editor. These sequences are used to generate a stable baseline before the experiment starts. The only difference between these sequences is the physical positions for the solutions used.

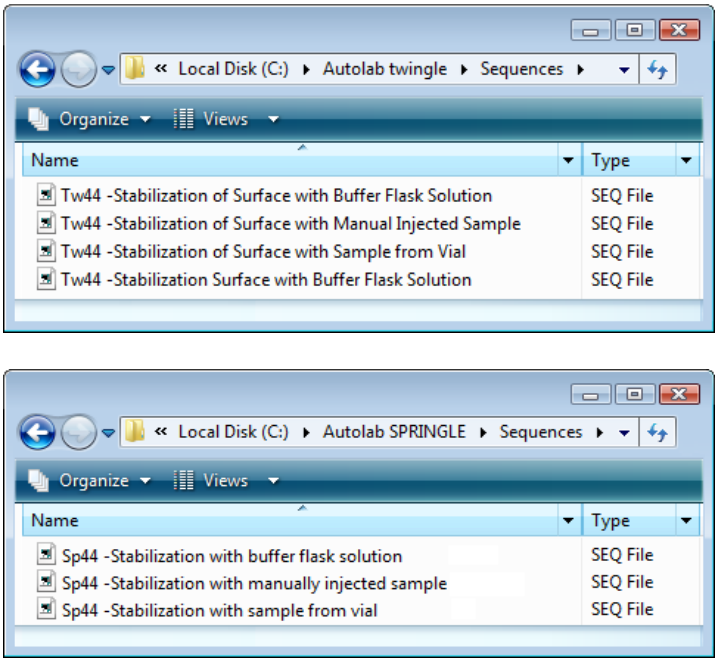


Figure 6.10 – List of stabilization sequences.

6.5.6.5 – The main immobilization sequence with all of its include-sequences.

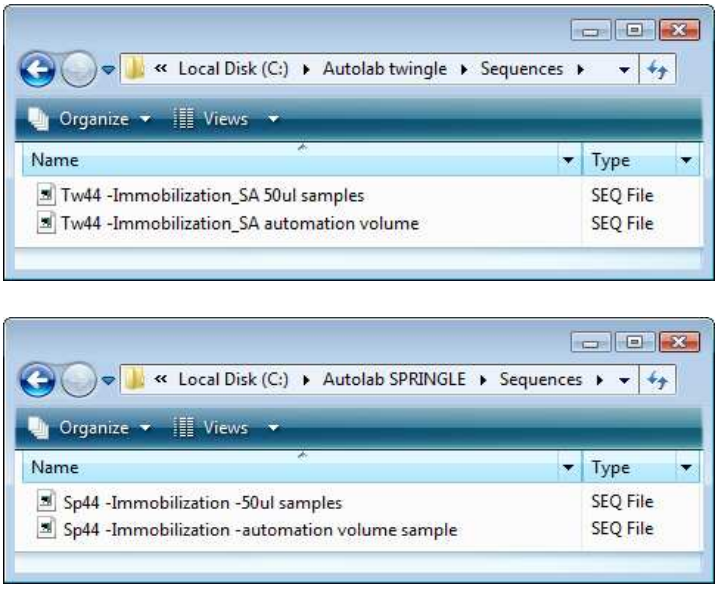


Figure 6.11 – The imobilization sequences.

The immobilization sequence is used to immobilize a ligand on the modified gold surface. The sequence in combination with the automation control

window will perform a chemically covalent binding using the EDC/NHS strategy. For other immobilization techniques, new sequences need to be written.
For the explanation of the automation the Twingle has been used as an example. The Springle has the same setup.

<table>
<tr><td>Tw44–Immobilization_SA 50µl samples.SEQ</td><td>Main sequence.</td></tr>
<tr><td></td><td></td></tr>
<tr><td>Tw44-Immobilization - Baseline with Coupling Buffer.SEQ</td><td rowspan="4">First level of include-sequences. Include-sequences inside the main sequence.</td></tr>
<tr><td>Tw44–Immobilization_SA 50 µl mixture EDC-NHS for activation step.SEQ</td></tr>
<tr><td>Tw44–Immobilization_SA Ligand Coupling step.SEQ</td></tr>
<tr><td>Tw44–Immobilization_SA Ethanol Amine Deactivation Step.SEQ</td></tr>
<tr><td colspan="2">Tw44-Immobilization - Regeneration Cleaning Step.SEQ</td></tr>
<tr><td></td><td rowspan="2">The include-sequence inside the above sequence sets the sample volume.</td></tr>
<tr><td>Tw44-Immobilization - inject 50µl Baseline Coupling Buffer.SEQ</td></tr>
</table>

Remark;
Instead of a long EDC/NHS activation time, it is better to have multiple incubation times with refreshed EDC/NHS solutions. For this the sequence needs two extra commands line. The include sequence “Tw44–Immobilization_SA 50 µl mixture EDC-NHS for activation step » needs a “Loop.Begin;Repeat=3” up front and the command “Loop.End” thereafter.

6.5.7 – The semi-automatic sequences.

In a semi-automatic sequence, the sample is introduced manually to the needles/pipette. The sample has to be presented manually to the pipette tip at the lift “up” position. In this position a sample vial can be put under the needles/pipette. The sample can be aspirated from the vial and dispensed into the cuvette. The semi-automatic sequences can be recognized by the abreviation SA (semi automatic) in the sequence name, for example “Tw44 - Curve - a full kinetic plot_SA 50µl sample.seq “

Two basic commands in the sequence make the experiment semi-automated;
1. Lift.Up in combination with Lift.Down
2. Message.Alert =[]

With the Lift.Up command the needle/pipette tip have direct access for a sample in a vial. The Message.Alert command the experiment stops the sequence and shows a “message box” window. The message shown has been written in the sequence, like….

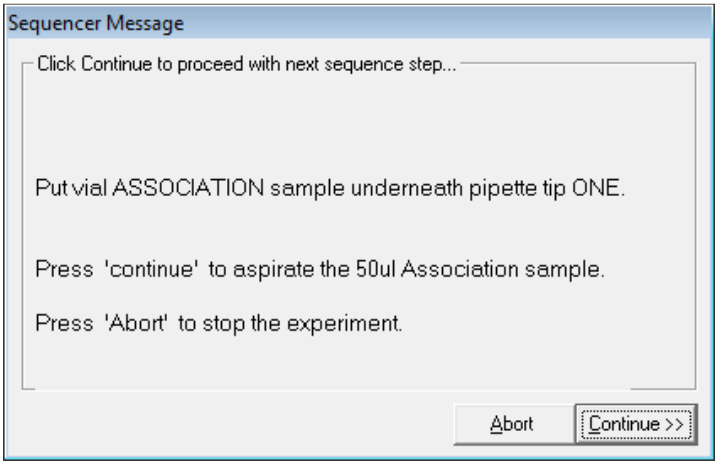

Sequencer Message
Click Continue to proceed with next sequence step...
Put vial ASSOCIATION sample underneath pipette tip ONE.
Press 'continue' to aspirate the 50ul Association sample.
Press 'Abort' to stop the experiment.
Abort
Continue >>

Figure 6.12 – Example of the sequence message alert box.

With the “continue” button, the sequence proceeds to aspirate the sample from the vial. A new message alert will pop up and request to remove the vial. Then after clicking the “continue” button the sequence will proceed with the experiment and start to measure.

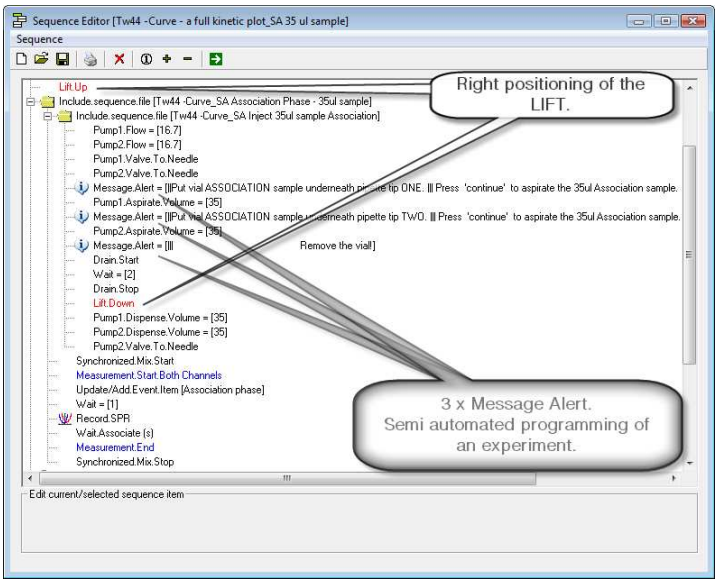


Figure 6.13 – An example of a sequence where the message alert has been used.

6.5.8 – Writing a sequence.

This section explains how to create a sequence to measure a binding curve. For the explanation of the automation the Twingle has been used as an example. The Springle has the same setup.

Basic routines of a binding curve:
1. baseline (buffer)
2. association phase (analyte)
3. dissociation phase (buffer)
4. regeneration phase (regeneration solution)
5. baseline (buffer)

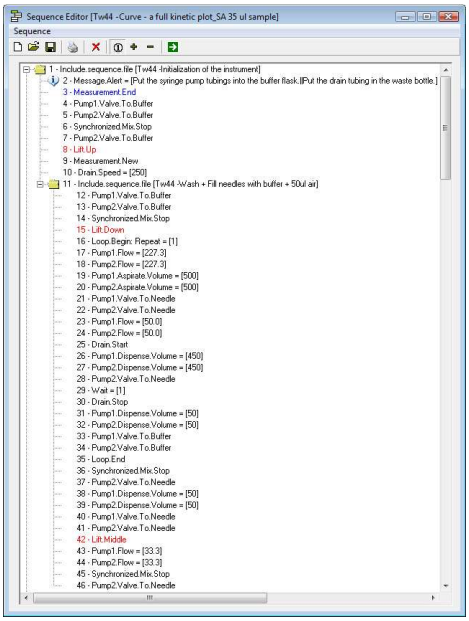
Every phase is written as an include-sequence. A commonly used include-sequence is the Parameters.seq. This sequence defines the measurement variables; flow speed, mix volume and measurement interval. Before starting a measurement it is advised to set the syringes in the home position and to specify all parameters.

Be careful not to contaminate the buffer in the tubing or syringes with sample, always use 50 µl of air between buffer in the needle/pipette tip tubing and sample.

Sequence order;

- Define measurement variables ▸ [PARAMETER.seq]
- Measures baseline ▸ [Tw44 -Curve -Baseline phase.seq]
- Baseline time ▸ Wait.baseline[s]
- Define sample position ▸ Sample.Move.To.Sample[], ▸ or Sampler.Next.⇒ If ready then step [])
- Inject sample and measure ▸ [Tw44 –Curve_SA Association phase. SEQ]
- Association time ▸ Wait.association[s]
- Dissociate complex ▸ [Tw44 -Curve -Dissociation phase.seq]
- Dissociation time ▸ Wait.dissociation[s]
- Regenerate surface ▸ [Tw44 –Curve_SA Regeneration phase.seq]
- Regeneration time ▸ Wait regeneration[s]
- Wash cuvette ▸ [Tw44 -Curve -Back to Baseline phase.seq
- End ▸ [END.seq]
- Conservation gold ▸ [Tw44 -Conservation quality of gold disk]

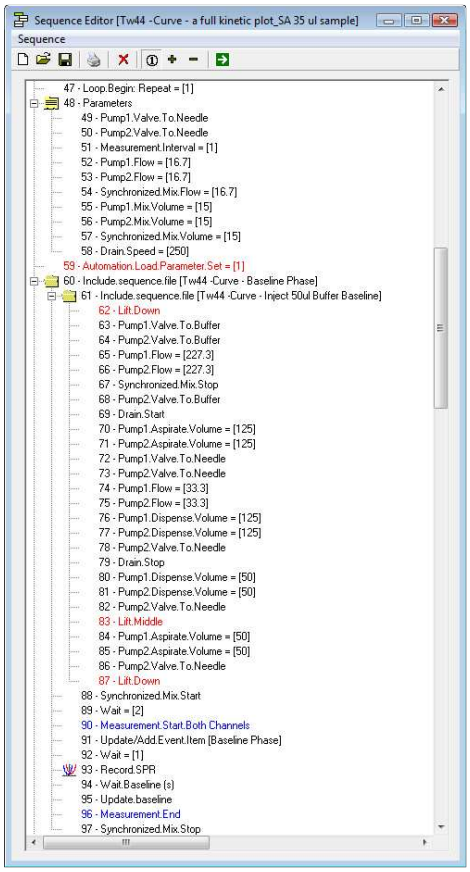
The measurement variables can either be defined in the sequence itself, or as a sequence variable that is defined in the automation window. Below an example of the sequence “Tw44 -Curve – a full kinetic plot_SA 50µl sample.seq” is explained.


Sequence Editor [Tw44 -Curve - a full kinetic plot_SA 35 ul sample]
Sequence
1 - Include.sequence.file [Tw44 -Initialization of the instrument]
2 - Message.Alert = [Put the syringe pump tubings into the buffer flask.|Put the drain tubing in the waste bottle.]
3 - Measurement.End
4 - Pump1.Valve.To.Buffer
5 - Pump2.Valve.To.Buffer
6 - Synchronized.Mix.Stop
7 - Pump2.Valve.To.Buffer
8 - Lift.Up
9 - Measurement.New
10 - Drain.Speed = [250]
11 - Include.sequence.file [Tw44 -Wash + Fill needles with buffer + 50ul air]
12 - Pump1.Valve.To.Buffer
13 - Pump2.Valve.To.Buffer
14 - Synchronized.Mix.Stop
15 - Lift.Down
16 - Loop.Begin: Repeat = [1]
17 - Pump1.Flow = [227.3]
18 - Pump2.Flow = [227.3]
19 - Pump1.Aspirate.Volume = [500]
20 - Pump2.Aspirate.Volume = [500]
21 - Pump1.Valve.To.Needle
22 - Pump2.Valve.To.Needle
23 - Pump1.Flow = [50.0]
24 - Pump2.Flow = [50.0]
25 - Drain.Start
26 - Pump1.Dispense.Volume = [450]
27 - Pump2.Dispense.Volume = [450]
28 - Pump2.Valve.To.Needle
29 - Wait = [1]
30 - Drain.Stop
31 - Pump1.Dispense.Volume = [50]
32 - Pump2.Dispense.Volume = [50]
33 - Pump1.Valve.To.Buffer
34 - Pump2.Valve.To.Buffer
35 - Loop.End
36 - Synchronized.Mix.Stop
37 - Pump2.Valve.To.Needle
38 - Pump1.Dispense.Volume = [50]
39 - Pump2.Dispense.Volume = [50]
40 - Pump1.Valve.To.Needle
41 - Pump2.Valve.To.Needle
42 - Lift.Middle
43 - Pump1.Flow = [33.3]
44 - Pump2.Flow = [33.3]
45 - Synchronized.Mix.Stop
46 - Pump2.Valve.To.Needle

Syringe starts in default middle position.
- Lines 4,5,67; set syringe pumps to middle
- Line 9; Cleans the DA window
- Line 11; fills/wash needle/pipette tip and peristaltic tubing;

- Lines 19,20; max. aspiration volume to fill up the 500ul syringe barrel.
- Lines 26,27; Dispenses volume to clean the needle/pipette tip and cuvette.
- Lines 31,32; dispense 50 µl on the gold surface
- Line 36; will put the syringe to middle position
- Lines 38,39 together with 42 and 45 aspirate 50 µl air into the needle/pipette tip.

SPECIALS, lines 7, 28, 37, 46 The command to the valve of the syringe is used to let the syringe finish its aspirate or dispense actions, before going to the next step in the sequence/experiment.

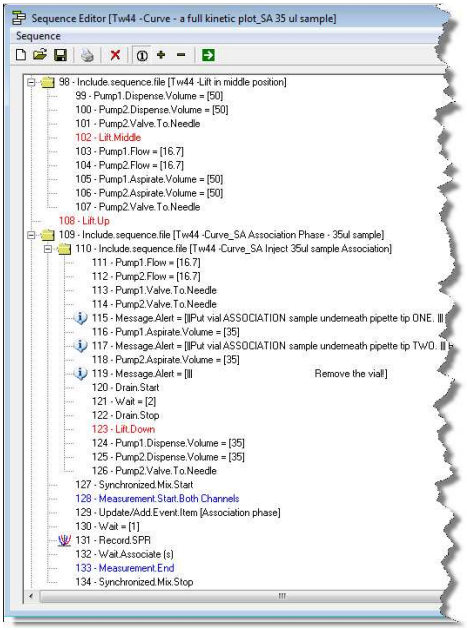

Sequence Editor [Tw44 -Curve - a full kinetic plot_SA 35 ul sample]
Sequence
47 - Loop.Begin: Repeat = [1]
48 - Parameters
49 - Pump1.Valve.To.Needle
50 - Pump2.Valve.To.Needle
51 - Measurement.Interval = [1]
52 - Pump1.Flow = [16.7]
53 - Pump2.Flow = [16.7]
54 - Synchronized.Mix.Flow = [16.7]
55 - Pump1.Mix.Volume = [15]
56 - Pump2.Mix.Volume = [15]
57 - Synchronized.Mix.Volume = [15]
58 - Drain.Speed = [250]
59 - Automation.Load.Parameter.Set = [1]
60 - Include.sequence.file [Tw44 -Curve - Baseline Phase]
61 - Include.sequence.file [Tw44 -Curve - Inject 50ul Buffer Baseline]
62 - Lift.Down
63 - Pump1.Valve.To.Buffer
64 - Pump2.Valve.To.Buffer
65 - Pump1.Flow = [227.3]
66 - Pump2.Flow = [227.3]
67 - Synchronized.Mix.Stop
68 - Pump2.Valve.To.Buffer
69 - Drain.Start
70 - Pump1.Aspirate.Volume = [125]
71 - Pump2.Aspirate.Volume = [125]
72 - Pump1.Valve.To.Needle
73 - Pump2.Valve.To.Needle
74 - Pump1.Flow = [33.3]
75 - Pump2.Flow = [33.3]
76 - Pump1.Dispense.Volume = [125]
77 - Pump2.Dispense.Volume = [125]
78 - Pump2.Valve.To.Needle
79 - Drain.Stop
80 - Pump1.Dispense.Volume = [50]
81 - Pump2.Dispense.Volume = [50]
82 - Pump2.Valve.To.Needle
83 - Lift.Middle
84 - Pump1.Aspirate.Volume = [50]
85 - Pump2.Aspirate.Volume = [50]
86 - Pump2.Valve.To.Needle
87 - Lift.Down
88 - Synchronized.Mix.Start
89 - Wait = [2]
90 - Measurement.Start.Both Channels
91 - Update/Add.Event.Item [Baseline Phase]
92 - Wait = [1]
93 - Record.SPR
94 - Wait.Baseline (s)
95 - Update.baseline
96 - Measurement.End
97 - Synchronized.Mix.Stop

The basic measurement routine is defined in a loop (line 42 till line 274), which is repeated 1 times.
Lines 48-58 define all parameters for the measurements, which is also done by
Line 59 which is linked to the automation window parameters,

The baseline include sequence
Lines 76,77; flush 125 µl buffer over the gold
Lines 80,81; define the baseline sample volume
Lines 84,85; take up 50 µl of air

Lines 88-97
A measurement has a fixed set of commands;
- start mixing, line 88
- start the measurement, line 90
- update/Add.Event.Item[], line 91
- update SPR, line 93
- incubation time, line 94
- end the measurement, line 96
- stop mixing, line 97

SPECIALS, lines 68, 78, 82, 86, the syringe finish its aspirate or dispense actions, before going to the next step in the sequence/experiment.

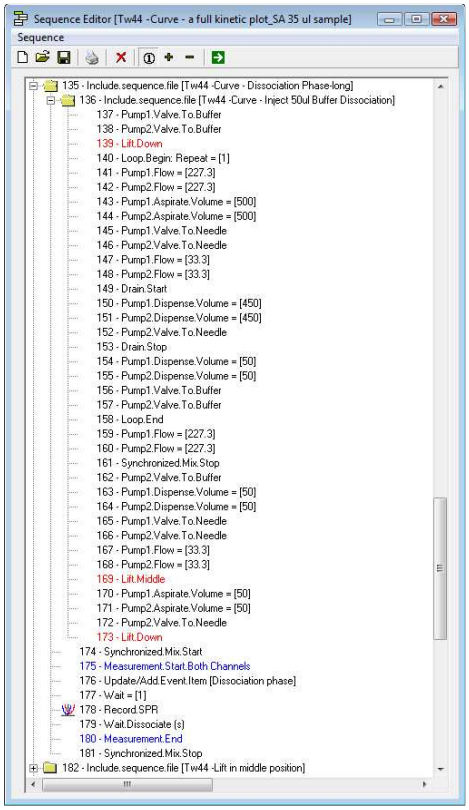

Sequence Editor [Tw44 -Curve - a full kinetic plot_SA 35 ul sample]
Sequence
98 - Include.sequence.file [Tw44 -Lift in middle position]
99 - Pump1.Dispense.Volume = [50]
100 - Pump2.Dispense.Volume = [50]
101 - Pump2.Valve.To.Needle
102 - Lift.Middle
103 - Pump1.Flow = [16.7]
104 - Pump2.Flow = [16.7]
105 - Pump1.Aspirate.Volume = [50]
106 - Pump2.Aspirate.Volume = [50]
107 - Pump2.Valve.To.Needle
108 - Lift.Up
109 - Include.sequence.file [Tw44 -Curve_SA Association Phase - 35ul sample]
110 - Include.sequence.file [Tw44 -Curve_SA Inject 35ul sample Association]
111 - Pump1.Flow = [16.7]
112 - Pump2.Flow = [16.7]
113 - Pump1.Valve.To.Needle
114 - Pump2.Valve.To.Needle
115 - Message.Alert = [||Put vial ASSOCIATION sample underneath pipette tip ONE. ||
116 - Pump1.Aspirate.Volume = [35]
117 - Message.Alert = [||Put vial ASSOCIATION sample underneath pipette tip TWO. ||
118 - Pump2.Aspirate.Volume = [35]
119 - Message.Alert = [|| Remove the vial!]
120 - Drain.Start
121 - Wait = [2]
122 - Drain.Stop
123 - Lift.Down
124 - Pump1.Dispense.Volume = [35]
125 - Pump2.Dispense.Volume = [35]
126 - Pump2.Valve.To.Needle
127 - Synchronized.Mix.Start
128 - Measurement.Start.Both Channels
129 - Update/Add.Event.Item [Association phase]
130 - Wait = [1]
131 - Record.SPR
132 - Wait.Associate (s)
133 - Measurement.End
134 - Synchronized.Mix.Stop

Line 98-107;
The include sequence has been written to be sure that the needle will have no sample left from previous actions.
Lines 108 to 123 were discussed in figure 5.13.

- Line 108; The needle/pipette tip is accessible to put a vial underneath
- Line 115, 117; Stop the experiment to put the vial underneath the needle/pipette tip
- Line 116, 118; Aspirate the sample from the vial
- Line 119; Stops the experiment to give time to remove the vial from under the needle/pipette tip
- Line 120-122; to drain away any solution on top of the gold before the next sample will be dispensed,

Lines 127 – 134; The fixed set of commands to perform the measurement.

SPECIALS, lines 101, 107, 126; The syringe finish its aspirate or dispense actions, before going to the next step in the sequence/experiment.

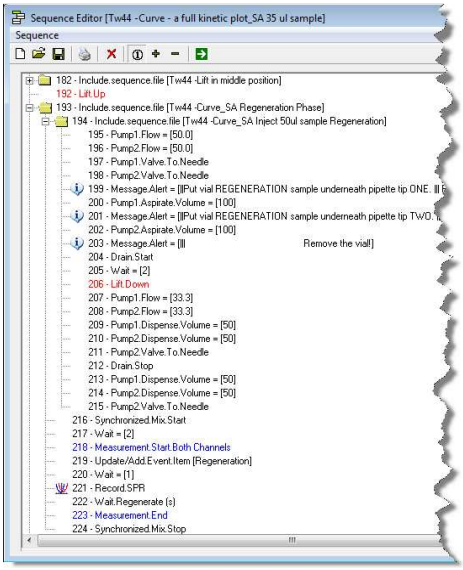

Sequence Editor [Tw44 -Curve - a full kinetic plot_SA 35 ul sample]
Sequence
135 - Include.sequence.file [Tw44 -Curve - Dissociation Phase-long]
136 - Include.sequence.file [Tw44 -Curve - Inject 50ul Buffer Dissociation]
137 - Pump1.Valve.To.Buffer
138 - Pump2.Valve.To.Buffer
139 - Lift.Down
140 - Loop.Begin: Repeat = [1]
141 - Pump1.Flow = [227.3]
142 - Pump2.Flow = [227.3]
143 - Pump1.Aspirate.Volume = [500]
144 - Pump2.Aspirate.Volume = [500]
145 - Pump1.Valve.To.Needle
146 - Pump2.Valve.To.Needle
147 - Pump1.Flow = [33.3]
148 - Pump2.Flow = [33.3]
149 - Drain.Start
150 - Pump1.Dispense.Volume = [450]
151 - Pump2.Dispense.Volume = [450]
152 - Pump2.Valve.To.Needle
153 - Drain.Stop
154 - Pump1.Dispense.Volume = [50]
155 - Pump2.Dispense.Volume = [50]
156 - Pump1.Valve.To.Buffer
157 - Pump2.Valve.To.Buffer
158 - Loop.End
159 - Pump1.Flow = [227.3]
160 - Pump2.Flow = [227.3]
161 - Synchronized.Mix.Stop
162 - Pump2.Valve.To.Buffer
163 - Pump1.Dispense.Volume = [50]
164 - Pump2.Dispense.Volume = [50]
165 - Pump1.Valve.To.Needle
166 - Pump2.Valve.To.Needle
167 - Pump1.Flow = [33.3]
168 - Pump2.Flow = [33.3]
169 - Lift.Middle
170 - Pump1.Aspirate.Volume = [50]
171 - Pump2.Aspirate.Volume = [50]
172 - Pump2.Valve.To.Needle
173 - Lift.Down
174 - Synchronized.Mix.Start
175 - Measurement.Start.Both Channels
176 - Update/Add.Event.Item [Dissociation phase]
177 - Wait = [1]
178 - Record.SPR
179 - Wait.Dissociate (s)
180 - Measurement.End
181 - Synchronized.Mix.Stop
182 - Include.sequence.file [Tw44 -Lift in middle position]

Line 136; Tw44 -Curve - Inject 50 µl buffer dissociation;
The time involves about 10 seconds before measurement starts. Replace this include sequence with a different file if this takes to long for the current application.

- Lines 150,151; flush 450 µl buffer to wash away the analyte,
- Line 154, 155; dispense the buffer sample to measure the dissociation
- Lines 156,157,159,160,161 to put the syringe in the middle position
- Lines 163,164, 170,171; aspirate 50 µl air. This to prevent contamination of the buffer in the tubing with the sample in the cuvette

Lines 174 to 181; The fixed set of commands to perform the measurement.
Line 182; The include sequence has been written to be sure that the needle will have no sample left from previous actions.

SPECIALS, lines 152,162,172


Sequence Editor [Tw44 -Curve - a full kinetic plot_SA 35 ul sample]
Sequence
182 - Include.sequence.file [Tw44 -Lift in middle position]
192 - Lift.Up
193 - Include.sequence.file [Tw44 -Curve_SA Regeneration Phase]
194 - Include.sequence.file [Tw44 -Curve_SA Inject 50ul sample Regeneration]
195 - Pump1.Flow = [50.0]
196 - Pump2.Flow = [50.0]
197 - Pump1.Valve.To.Needle
198 - Pump2.Valve.To.Needle
199 - Message.Alert = [[Put vial REGENERATION sample underneath pipette tip ONE.
200 - Pump1.Aspirate.Volume = [100]
201 - Message.Alert = [[Put vial REGENERATION sample underneath pipette tip TWO.
202 - Pump2.Aspirate.Volume = [100]
203 - Message.Alert = [[Remove the vial!]
204 - Drain.Start
205 - Wait = [2]
206 - Lift.Down
207 - Pump1.Flow = [33.3]
208 - Pump2.Flow = [33.3]
209 - Pump1.Dispense.Volume = [50]
210 - Pump2.Dispense.Volume = [50]
211 - Pump2.Valve.To.Needle
212 - Drain.Stop
213 - Pump1.Dispense.Volume = [50]
214 - Pump2.Dispense.Volume = [50]
215 - Pump2.Valve.To.Needle
216 - Synchronized.Mix.Start
217 - Wait = [2]
218 - Measurement.Start.Both Channels
219 - Update/Add.Event.Item [Regeneration]
220 - Wait = [1]
221 - Record.SPR
222 - Wait.Regenerate (s)
223 - Measurement.End
224 - Synchronized.Mix.Stop

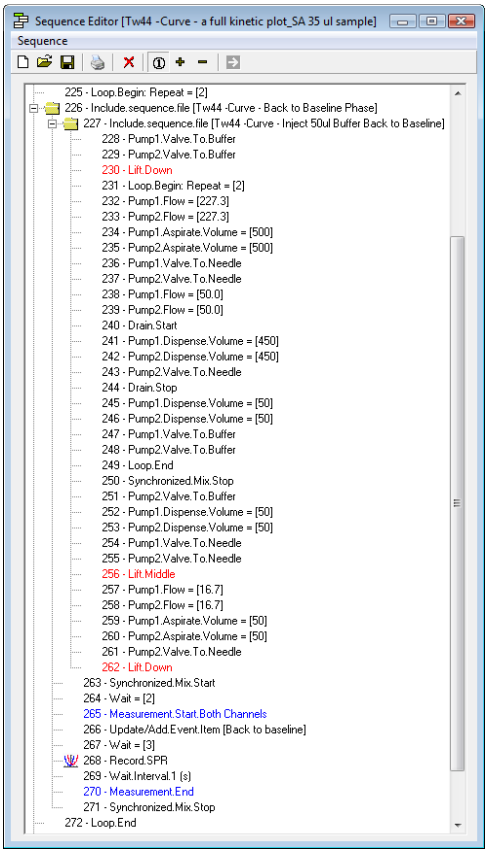
Line 182; The include sequence has been written to be sure that the needle will have no sample left from previous actions.

Lines 192 to 215 are similar to as lines 108-126.

- Line 192; The needle/pipette tip is accessible to put a vial underneath
- Line 199, 201; Stop the experiment to put the vial underneath the needle/pipette tip
- Line 200, 202; Aspirate the sample from the vial
- Line 203; Stops the experiment to give time to remove the vial from under the needle/pipette tip
- Line 204,205,212; to drain away any solution on top of the gold before the next sample will be dispensed,

Lines 216 – 224; The fixed set of commands to perform the measurement.

SPECIALS, lines 211, 215 The syringe finishes its aspirate or dispense actions, before going to the next step in the sequence/experiment.

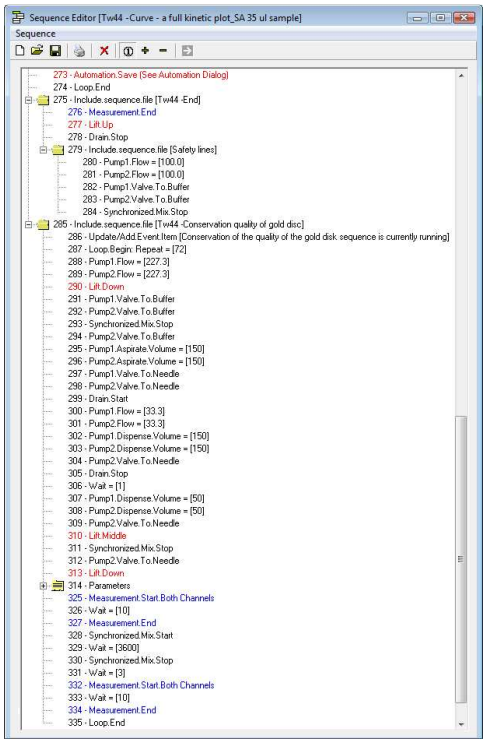

Sequence Editor [Tw44 -Curve - a full kinetic plot_SA 35 ul sample]
Sequence
225 - Loop.Begin: Repeat = [2]
226 - Include.sequence.file [Tw44 -Curve - Back to Baseline Phase]
227 - Include.sequence.file [Tw44 -Curve - Inject 50ul Buffer Back to Baseline]
228 - Pump1.Valve.To.Buffer
229 - Pump2.Valve.To.Buffer
230 - Lift.Down
231 - Loop.Begin: Repeat = [2]
232 - Pump1.Flow = [227.3]
233 - Pump2.Flow = [227.3]
234 - Pump1.Aspirate.Volume = [500]
235 - Pump2.Aspirate.Volume = [500]
236 - Pump1.Valve.To.Needle
237 - Pump2.Valve.To.Needle
238 - Pump1.Flow = [50.0]
239 - Pump2.Flow = [50.0]
240 - Drain.Start
241 - Pump1.Dispense.Volume = [450]
242 - Pump2.Dispense.Volume = [450]
243 - Pump2.Valve.To.Needle
244 - Drain.Stop
245 - Pump1.Dispense.Volume = [50]
246 - Pump2.Dispense.Volume = [50]
247 - Pump1.Valve.To.Buffer
248 - Pump2.Valve.To.Buffer
249 - Loop.End
250 - Synchronized.Mix.Stop
251 - Pump2.Valve.To.Buffer
252 - Pump1.Dispense.Volume = [50]
253 - Pump2.Dispense.Volume = [50]
254 - Pump1.Valve.To.Needle
255 - Pump2.Valve.To.Needle
256 - Lift.Middle
257 - Pump1.Flow = [16.7]
258 - Pump2.Flow = [16.7]
259 - Pump1.Aspirate.Volume = [50]
260 - Pump2.Aspirate.Volume = [50]
261 - Pump2.Valve.To.Needle
262 - Lift.Down
263 - Synchronized.Mix.Start
264 - Wait = [2]
265 - Measurement.Start.Both Channels
266 - Update/Add.Event.Item [Back to baseline]
267 - Wait = [3]
268 - Record.SPR
269 - Wait.Interval.1 (s)
270 - Measurement.End
271 - Synchronized.Mix.Stop
272 - Loop.End

- Lines 225,272; Define a loop (2x) for measuring the baseline twice.
- Lines 231 249; Define a loop (2x) to wash the gold twice.
- Line 227; Tw44 -Curve - Inject 50 µl buffer back to baseline;
  Aspirates 500ul buffer from flask to wash the needles with 500ul and subsequently 50 µl is used for the incubation.

- Lines 241,242; flush 450µl buffer to wash away the analyte,
- Lines 245, 246; the buffer sample to measure the dissociation
- Lines 247,248,250 to put the syringe in the middle position
- Lines 252,253, 259,260; aspirate 50 µl air. This to prevent contamination of the buffer in the tubing with the sample in the cuvette

Lines 263 to 271; The fixed set of commands to perform the measurement.

SPECIALS, lines 243, 251, 261 The syringe finishes its aspirate or dispense actions, before going to the next step in the sequence/ experiment.


Sequence Editor [Tw44 -Curve - a full kinetic plot_SA 35 ul sample]
Sequence
273 - Automation.Save (See Automation Dialog)
274 - Loop.End
275 - Include.sequence.file [Tw44 -End]
276 - Measurement.End
277 - Lift.Up
278 - Drain.Stop
279 - Include.sequence.file [Safety lines]
280 - Pump1.Flow = [100.0]
281 - Pump2.Flow = [100.0]
282 - Pump1.Valve.To.Buffer
283 - Pump2.Valve.To.Buffer
284 - Synchronized.Mix.Stop
285 - Include.sequence.file [Tw44 -Conservation quality of gold disc]
286 - Update/Add.Event.Item [Conservation of the quality of the gold disk sequence is currently running]
287 - Loop.Begin: Repeat = [72]
288 - Pump1.Flow = [227.3]
289 - Pump2.Flow = [227.3]
290 - Lift.Down
291 - Pump1.Valve.To.Buffer
292 - Pump2.Valve.To.Buffer
293 - Synchronized.Mix.Stop
294 - Pump2.Valve.To.Buffer
295 - Pump1.Aspirate.Volume = [150]
296 - Pump2.Aspirate.Volume = [150]
297 - Pump1.Valve.To.Needle
298 - Pump2.Valve.To.Needle
299 - Drain.Start
300 - Pump1.Flow = [33.3]
301 - Pump2.Flow = [33.3]
302 - Pump1.Dispense.Volume = [150]
303 - Pump2.Dispense.Volume = [150]
304 - Pump2.Valve.To.Needle
305 - Drain.Stop
306 - Wait = [1]
307 - Pump1.Dispense.Volume = [50]
308 - Pump2.Dispense.Volume = [50]
309 - Pump2.Valve.To.Needle
310 - Lift.Middle
311 - Synchronized.Mix.Stop
312 - Pump2.Valve.To.Needle
313 - Lift.Down
314 - Parameters
325 - Measurement.Start.Both Channels
326 - Wait = [10]
327 - Measurement.End
328 - Synchronized.Mix.Start
329 - Wait = [3600]
330 - Synchronized.Mix.Stop
331 - Wait = [3]
332 - Measurement.Start.Both Channels
333 - Wait = [10]
334 - Measurement.End
335 - Loop.End

- Line 273: saves the experiment with a file name defined in the automation window
- Line 274; finish the experiment
- Line 275; to be sure all actions are stopped

- Lines 285-335; Preserve the quality of the molecules attached to the gold surface.
- Lines 295,296; flush 150µl buffer to wash the surface,
- Lines 299, 305, drain away the solution from the gold
- Lines 307,308; the buffer sample to measure the baseline
- Lines 311; aspirate 50 µl air. This to prevent contamination of the buffer in the tubing with the sample in the cuvette

Every hour the solution will be replace by a new fresh solution

- Line 329; 1 hour of incubation
- Line 326,333; wait 10 seconds to measure the SPR signal
- Lines 287,335; 72 repeats of this procedure, cover 72 hours of conserving the gold surface.

Chapter 7

7 – Automation

7.1 – Index.

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7 – Automation 142
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7.2 – Introduction.

The Automation allows the user to perform measurements semi-automatically, with customized variables such as times, volumes and other parameters. Every issue of the automation control window will only be used if the sequence to be executed has the commands which will be linked to the automation control window (see chapter 6).

7.3 – How to open the Automation Control Window.

The automation control window is activated by selecting the automation button at the tool bar or under menu <options> (Figure 7.1). For the explanation of the automation the Twingle has been used as an example. The Springle has the same setup.

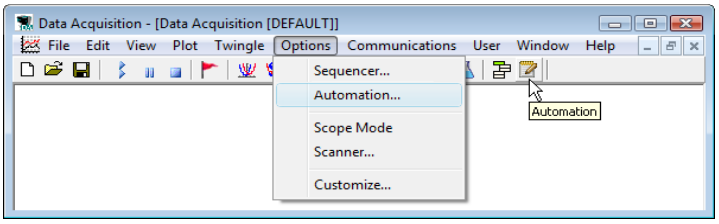

Figure 7.1 – The autosampler control window selection.

The automation control window consists of a number of selection windows.

- Parameters, times and volumes tab sheets.
- File name.
- Execute button.

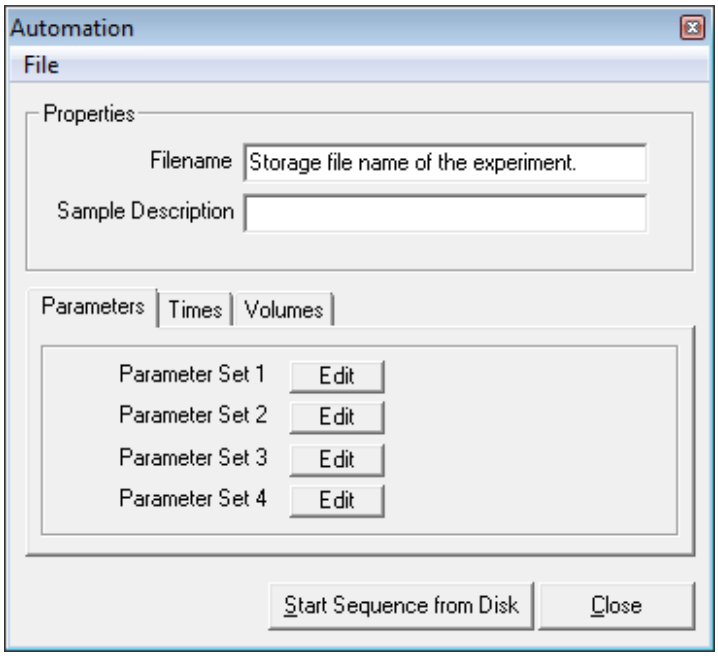

Figure 7.2 – The automation window with three tab sheets to set up the experiment.

Although the name of the window suggests the experimental control is automatic, measurements can only be performed semi-automatic. Whenever a sample needs to be introduced to the gold surface, this action needs manually presenting the sample vial to the needles/pipette tips.

7.4 – The Automation control window.

The automation window enables changing parameters, incubation times and sample volume outside of the sequence editor. Specific sequence commands are linked to a typical box that can be filled out in the Automation window.

The sequencer command "Automation.Load.Parameter.Set =[1-4]" will look for the parameters settings filled out in the parameters TAB sheet (figure 6.2). With the button EDIT, a new window "System parameters" pops up. Within this window every item can be adjusted. The settings will be used for the experiment and loaded into the sequence whenever the command line "Automation.Load.Parameters.Set = [1-4]" is written.

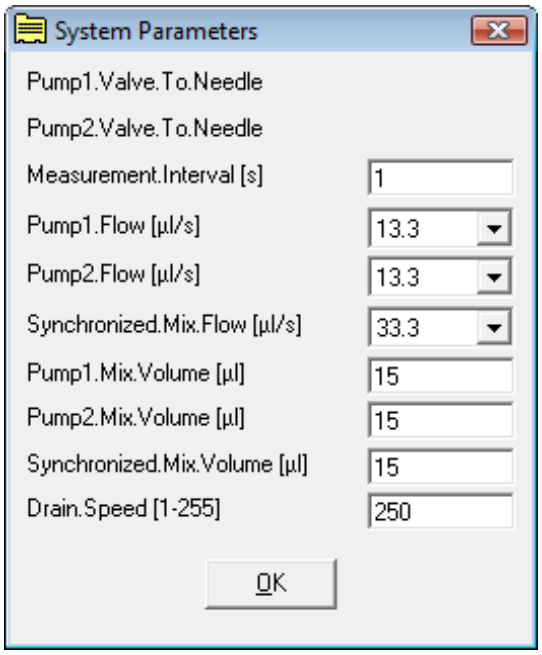

Figure 7.3 – The EDIT button gives access to these settings.

When the desired parameters settings are set, the incubation times can be adjusted.

Select the “Times” Tab sheet to address the incubation times of the experiment. Eight different times can be edited. The box to edit depends on the sequence to be executed. So, the typical “WAIT.xxxxxx [s]” (§6.5.6) used in the sequence decides which box is necessary to fill out. Tables 6.1 and 6.2 show some examples.

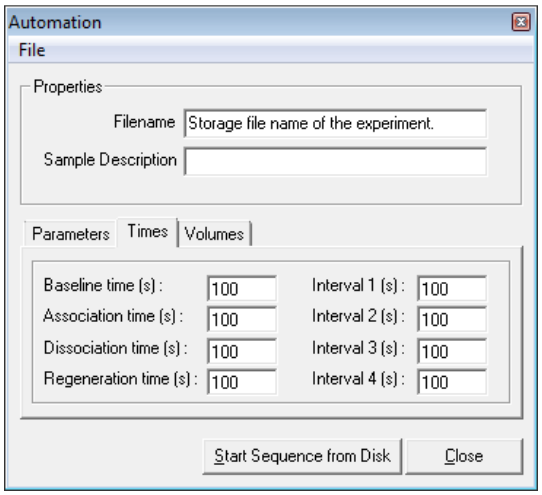

Figure 7.4 – The automation window with the incubation time TAB sheet to set up the experiment.

Last but not least, whenever the commands “Automation.Pump.Aspirate” and “Automation.Pump.Dispense” are used in the sequence, the Tab sheet Volumes needs to be filled out.

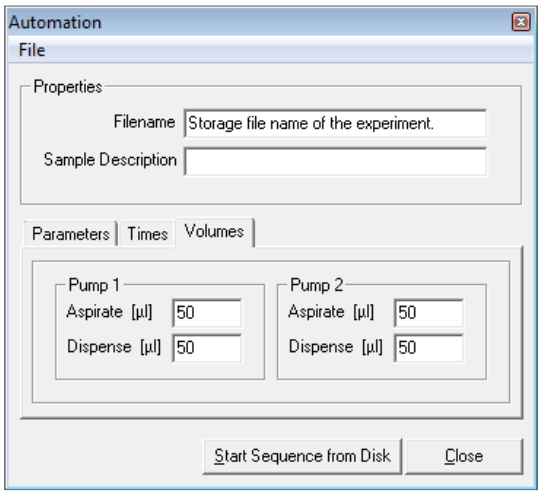

Figure 7.5 – The automation window with the volumes time TAB sheet to set up the sample volumes of the experiment.

Chapter 8

8 – SPR theory.

8.1 – Index.

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8 – SPR theory. 146
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8.4.1 – Optics of the Twingle system. 154
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8.4.3 – Cuvette. 159
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8.5 – SPR methods. 161
8.5.1 – Introduction. 161
8.5.2 – Methods using the SPR disk. 161
8.6 – References. 162

8.2 – Introduction.

A biosensor is a device that incorporates a biological recognition (sensing) element in close proximity to, or integrated with the signal transducer, to give a reagentless sensing system specific to a target compound (analyte). Transducers are the physical components of the sensor that react to a signal due to the interaction between the biological sensing element and the target analyte. Biosensing occurs only when the analyte is recognized specifically by the biological element. The biological recognition elements can be divided into two distinct groups: catalytic and non-catalytic. The catalytic group includes enzymes, micro-organisms and plant or mammalian tissue, while the non-catalytic or affinity class includes antibodies, receptors, and nucleic acids. The interaction output can be amplified, stored, or displayed.
The advantage of a biosensor is the label-free detection of the interaction. The label-free form in most cases is achieved by immobilizing the biological recognition element. In general, the immobilization matrix may function purely as a support, or else, it may also be concerned with mediation of the signal transduction mechanism associated with the analyte. Immobilization techniques include physical entrapment by an inert membrane, physical/chemical adsorption, binding to a functionalised support, and entrapment in an 'active' membrane.

Surface Plasmon Resonance is a specific biosensor, a special case of the interaction of light with matter. SPR signals are related to the refractive index close to the sensor surface, and are therefore related to the amount of macromolecules bound to the sensor surface.

Biomolecular interactions are conventionally studied by techniques as immunoassays (ELISA or RIA), equilibrium dialysis, affinity chromatography and spectroscopic techniques. The main advantage of SPR over these techniques to study biomolecular interactions is real-time monitoring of binding events and label-free detection of macromolecular interactions. Advantages of the Twingle instrument are the modular-set up, which enables a flexible design of experiments, and rapid analysis of the interaction plots by the kinetic evaluation software. Interaction plots will show binding curves of macromolecular interactions and baseline shifts due to changes in refractive indices of sample solutions. Information can be obtained from the binding curves, which include properties like:

- Specificity — Which molecules interact?
- Concentration — How many molecules are there?
- Affinity — How strong is the interaction?
- Kinetics — How fast is the interaction?
- Cooperativity — Are there any steric/allosteric effects?
- Biocompatibility/coatings — How does a molecule interact with a coating?

8.3 – Surface Plasmon Resonance.

Surface plasmons are created by a consistent longitudinal charge fluctuation at the surface of a metal and typically have their intensity maximum in the surface and exponentially decaying field perpendicular to it. The surface plasmon is a p(plane)-polarized surface bound electromagnetic wave propagating at the interface between a metal and a dielectric.

Our SPR system measurement principle.

Surface plasmon resonance[1-4] occurs under certain conditions when a thin film of metal (gold or silver) is placed inside the laser beam. When the incoming light is monochromatic and p-polarized (i.e. the electric vector component is parallel to the plane of incidence), the free electrons of the metal will oscillate and absorb energy at a certain angle of incident light. The angle of incidence at which SPR occurs is called the SPR angle. SPR is detected by measurement of the intensity of the reflected light. At the SPR angle, a sharp decrease or 'dip' in intensity is measured. The position of the SPR angle depends on the refractive index in the substance with a low-refractive index, i.e. the sensing surface.
The refractive index of the sensor surface changes upon binding of macromolecules to the surface. As a result, the SPR wave will change and therefore the angle will change accordingly. There is a linear relationship between the amount of bound material and shift in SPR angle[5]. The SPR angle shift in millidegrees is used as a response unit to quantify the binding of macromolecules to the sensor surface. The response also depends on the refractive index of the bulk solution. A change of 122 millidegrees represents a change in surface protein of approximately 1 ng/mm^2, or in bulk refractive index of approximately 10^{-3}.

Table 8.1. Correlation of SPR parameters

SPR parameters	Equivalent values
SPR angle shift	122 millidegrees
Change in protein surface concentration	1 ng/mm^2
Change in bulk refractive index	0.001

The detection principle limits the size of the analyte, which can be studied. If the molecular weight of the compound is below 1000 Dalton, then the change in refractive index upon binding to the sensor surface is too low to be detected directly. The penetration depth of the evanescent wave of 300-400 nm also determines the size of macromolecules or particles that can be studied. Particles larger than 400 nm cannot be measured totally. As a result, the signal is not linearly related to the amount of bound particles. Under these circumstances it is possible to study the binding qualitatively, but a quantitative or kinetic analysis cannot be performed.

Background information, history.

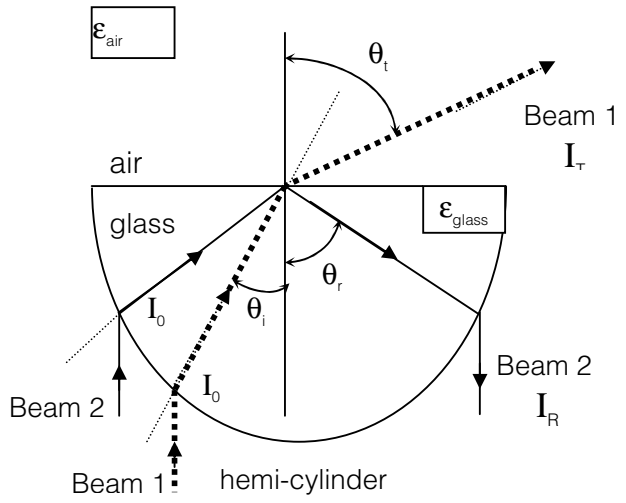
Augustin Fresnel presented in 1821-32 theories that, in principle, could have explained the SPR-phenomenon. James Clark Maxwell presented in 1873 all theories necessary to model SPR. He introduced the displacement current and wrote down the relations between the electric and magnetic fields, now known as the Maxwell's Equations. Loss of light incident onto a grating was first observed by R.W. Wood in 1902 while he was studying diffracted spectra of metallic gratings (Woods anomalies). In 1941 Frano suggested an excitation of electromagnetic surface waves. Pines and Bohm assumed that the observed energy losses were due to the excitation of plasma oscillations or "plasmons" of the conducting electrons (1951). This was in 1957 theoretically explained as Surface Plasmons (SP) by Ritchie. This theory was confirmed experimentally in reflection studies by Powell and Swan (1959). Later, Stern and Ferrell, observed "significant effect" on the angle of incidence at which energy losses occurred (1960). Turbadar presented experimental results of the SPR-phenomena and showed that it could be predicted by the thin film theory, 1968. Otto invented the attenuated total reflection (ATR) method to excite a surface plasmon. Between the prism and metal layer is a layer with air. In 1971 the method was improved by Kretschmann by applying a thin metal film directly onto an ATR prism, denoted the Kretschmann configuration. The Kretschmann configuration is the most used configuration, which is also the basic configuration of the Autolab Twingle instrument (Figure 8.3).

Theory.

Surface Plasmon Resonance is a physical process, which occurs when light hits a metal under a special angle position during total internal reflection conditions.

If a light beam passes the glass of a hemi cylinder prism, its path (angle) is changed when it leaves the prism into air (beam 1, Figure 8.1). This change always occurs when light passes through a denser medium into a less dense medium (or vice versa). At a critical angle of incidence, the light beam (beam 2, Figure 7.1) does not leave the prism, but will be reflected at the interface of the two media glass and air. This is called total internal reflection.

In the SPR situation we have a replaceable glass disk, coated with a thin layer of gold, on the hemi-cylinder. Between the disk and the hemi-cylinder is a thin layer of oil. The refractive index of the hemi-cylinder, the oil and the disk is the same. In this way the laser light will not bend passing the hemi-cylinder, the oil and the glass to reach the gold layer. The photons hit the gold instead of air at the total internal reflection angle.



Legend
Beam 1 is a refracted beam
Beam 2 is a reflected beam

I_O = intensity incoming light
I_R = intensity reflected light
I_T = intensity refracted light

θ_i = angle of incident light
θ_r = angle of reflected light
θ_t = angle of refracted light
$\theta_{i,\ beam\ 2} = \theta_{r,\ beam\ 2}$

ε_{air} = refractive index of air = ε_1
ε_{glass} = refractive index of glass = ε_2

in general : $\varepsilon_{air} < \varepsilon_{glass}$

Figure 8.1 – An overview of light beams passing through the hemi-cylinder of glass.

There is a special situation for the photons when a dielectric medium is placed on top of the gold.
If the dielectric medium has an opposite (or higher) dielectric constant than gold, the free electrons in the gold will fluctuate. This electron fluctuation gives charge fluctuations in the metal. The metal layer is very thin and therefore the charge fluctuations are only taking place at the surface and cause an electromagnetic surface wave, called surface plasma oscillations (Ritchie, 1957).

Remarks;

- Definition of plasma; plasma is a medium with equal concentration of positive and negative charges, of which at least one charge type (*e.g.* electron) is mobile (*e.g.,* metal).

- Metals are conductors of electricity and insulators (like plastics, glass) are called dielectrics.
  All kind of components, liquid, gasses, metals and salts have a dielectric constant.

- Electron charge fluctuations are possible in the volume of a plasma and in its boundary with a dielectric. The SPR situation has only the boundary electron charge fluctuation, because the metal layer is very thin.

- In physics photons and electrons are described as waves and particles properties. A plasmon is the particle name for the electron density waves.

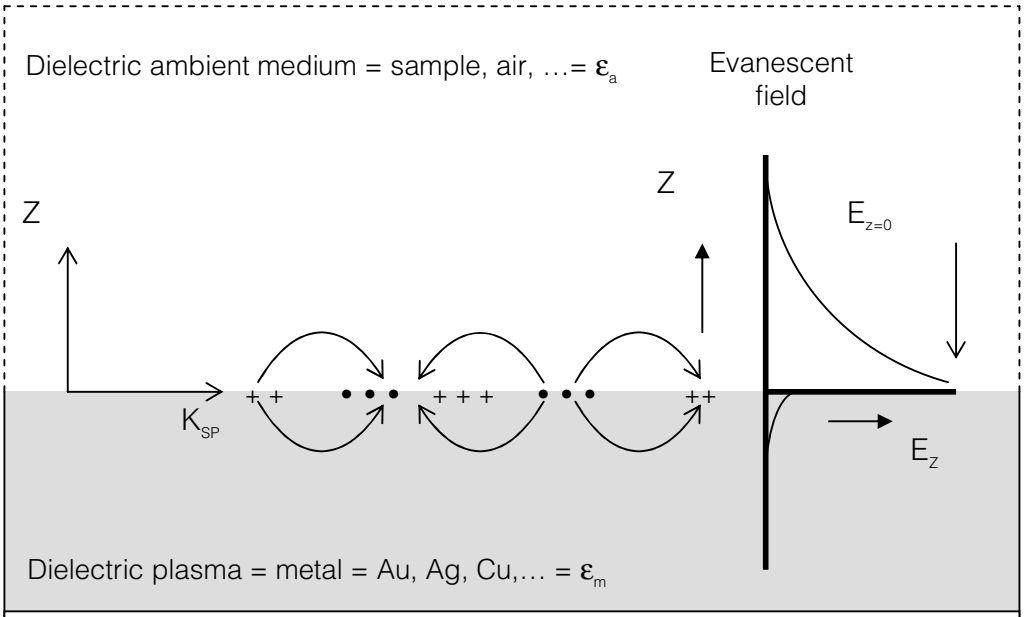


Figure 8.2 – Electron fluctuation.
Electron fluctuations give rise to a surface plasmon wave.
The generated evanescent field energy is maximum on the surface and decaying exponentially in the Z direction.

When light hits the gold at a certain angle of incidence, the energy of the photon can interact with the free fluctuating electron in the gold surface. In general the electromagnetic wave phenomenon, surface plasmon, can be excited by the fields of charged particles and photons. In our case, the surface plasmon is excited by photons. This is called surface plasmon resonance.

Therefore, when in the total internal reflection situation the energy of the light is ideal (SPR situation) and the photons are converted to (resonating) plasmons there will be (almost) no reflected light to detect by the detector. Plotting the light intensity versus angle of incidence will give a dip at the specific SPR angle.

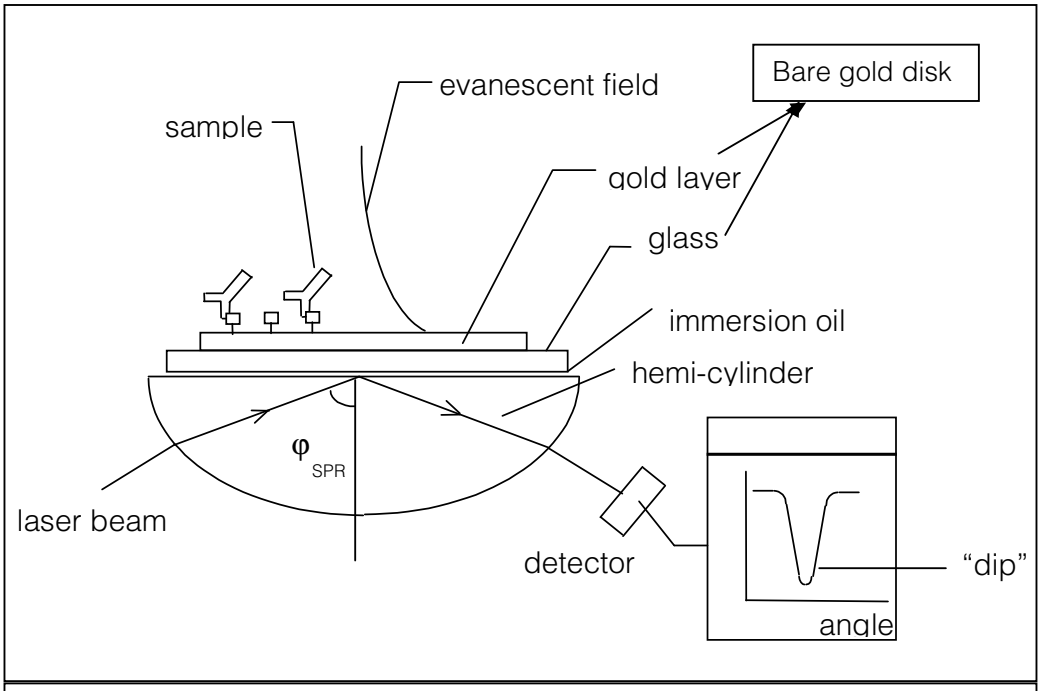


Figure 8.3 – Kretschmann configuration; special is the oil between hemi-cylinder and gold disk.

A incoming light beam is being reflected and detected by the detector. At a certain angle the reflected light intensity is decreased, at this point the Surface Plasmon Resonance effect occurs.

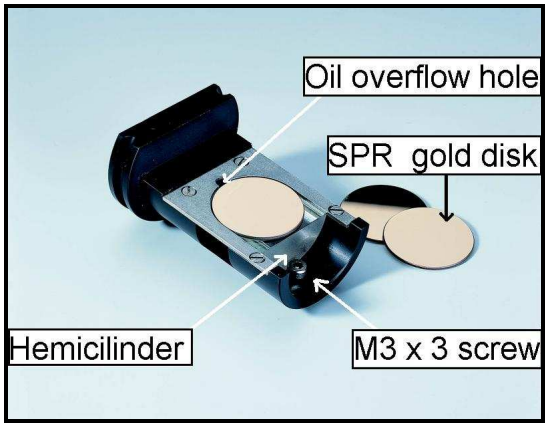


Figure 8.4 – Slider with hemi-cylinder.

8.4 – AUTOLAB Twingle configuration.

The Autolab Twingle is configured as a flexible instrument controlled by a computer that can be configured to individual needs. It is mainly composed of three parts (Figure 8.5):

Optics	Surface Plasmon Resonance is generated by vibrating mirror optics
Sensor	One gold-coated glass surface can be installed as sensor. The cuvette separates two areas to monitor two macromolecular interactions at the same time.
Liquid handling	The instrument is equipped with a continuously mixed cuvette

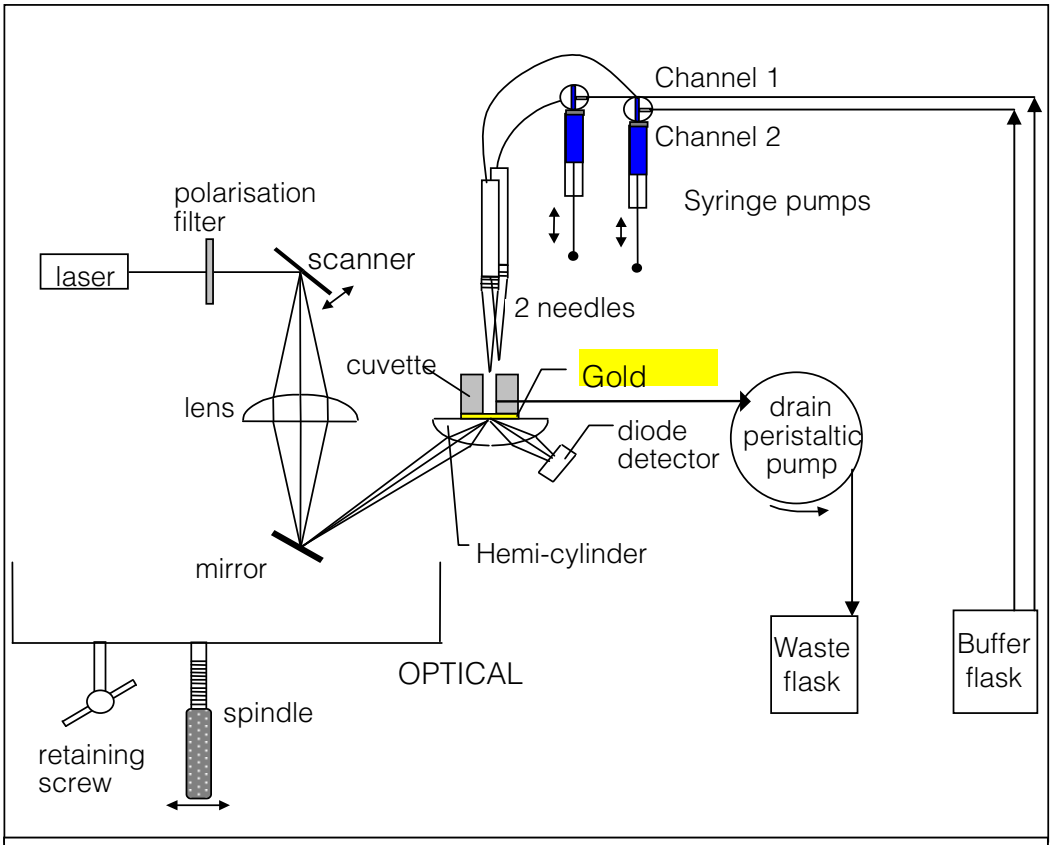


Figure 8.5 – Schematic picture of the TWINGLE configuration.

8.4.1 – Optics of the Twingle system.

The intensity of the reflected light (p-polarized with a wave length of 670 nanometer) is measured over a range of 4000 millidegrees. A scanning mirror with a frequency of 76 Hertz is used to obtain an angle scan of 4000 millidegrees in approximately 13 milliseconds. The SPR angle of a buffer solution can be fixed manually by a spindle with an offset SPR angle of 62°-78° degrees, which corresponds to a refractive index range of 1.33-1.43 of the sensor surface. The SPR angle scan is performed around the manually fixed SPR position.
The optical reflectance of incident light at different angles around the fixed SPR angle for a buffer solution is measured, while the laser beam is kept at one spot of the sensor surface of approximately 2 mm^2. This is accomplished by applying cylindrical optics. A half cylinder is used as a prism for the optical contact with the sensor surface. The optics are designed in such a way that a parallel light beam will be inside the half cylinder while scanning. The function of the cylindrical lens is two-fold. It projects the rotating axis of the vibrating mirror at the centre of the hemi-cylinder and compensates its converging effect.
The advantage of the Twingle optical configuration is that unwanted defects in the ligand specific layer will be averaged and artefacts due to spatial inhomogeneties are eliminated. The optical configuration results in an accurate, reproducible and sensitive detection.

In the vibrating mirror set-up[7], the angular shift is measured for a non-coated gold sensor surface with a resolution of approximately 0.05 millidegrees (m°), corresponding to a refractive index resolution of approximately $1*10^{-5}$. For a coated gold sensor surface, the angular shift is measured with a resolution of approximately 0.1 millidegrees (m°).

8.4.2 – Sensor.

Desirable features of the sensor surface for the study of macromolecular interactions are:

- A rapid, simple and reproducible immobilization technique
- Stability and retained biological activity of the immobilized biomolecules
- Low non-specific interaction
- Facilities for regeneration after use
- Flexibility in design of coatings for polymer-macromolecule interactions
- Possibilities to detect particles as viruses, bacteria and cells

These features cannot be combined in one sensor surface. For this reason, ESPR measurements can be performed using many different sensor surfaces. Measurements can be performed using a disk covered either with a bare gold layer or with a disk covered with one of the many options of modified gold layers.

The disk contains a gold layer of approximately 50 nm and is used to study interactions of coatings with macromolecules, and to study the interactions of large particles as viruses, bacteria and cells to coated proteins. A modified gold layer disk can be bought but also made with help of an Autolab spincoater. For example a thin film of polymer can be easily attached to the gold surface by the use of an Autolab spincoater.
An example of a commercially available modified gold disk is the dextran hydrogel modification. The hydrogel covering the gold surface of the sensor chip is composed of non-cross-linked carboxymethylated dextran, attached to the gold molecules via a thiol linker layer[8]. Dextran is a linear polymer of glucose units, which possesses very low non-specific adsorption of biomolecules. The dextran on the sensor chip is carboxymethylated, with a composition of one carboxyl group per glucose residue. Three purposes are achieved by the modification of dextran:

- Incorporation of a functional group for immobilization procedures of biomolecules

- Negatively charged polymer at physiological pH values, which allows positively charged biomolecules to adsorb electrostatically to the dextran layer under conditions of low ionic strength

- Enhancement of the hydrophilicity of the dextran layer by incorporation of carboxymethyl groups

Biomolecules can be immobilized by reaction with activated carboxymethyl functional groups of the dextran layer. Two functional groups of the ligand can be used for the immobilization. Ligands are coupled by amine functional groups or by thiol functional groups[9].
The dextran coating is very suitable to study a variety of macromolecular interactions. It is especially useful in the determination of kinetic parameters or antibody concentration. Three methods are used to couple ligands:

- Immobilization of the ligand to the dextran layer. The ligand is coupled covalent either by amine functional groups or by thiol functional groups to the dextran layer.
  Immobilization by amine functional groups of ligands is performed in three steps:
  1. reaction of carboxymethyl groups with a mixture of *N*-hydroxysuccinimide (NHS) and *N*-ethyl-*N*'-(dimethylaminopropyl)carbodimide (EDC) to obtain an active NHS ester
  2. reaction of the activated ester with primary amine functional groups of the ligand for a covalent ligand-hydrogel bond
  3. deactivation of excess activated ester groups with ethanolamine

- Non-covalent binding of biotinylated ligand to a streptavidin-immobilized gold disk. Due to the severe biotin-streptavidin interaction, it is possible to regenerate the surface without disrupting

the non-covalent biotin-streptavidin bond. This method is suitable to couple synthetic DNA molecules to the surface.

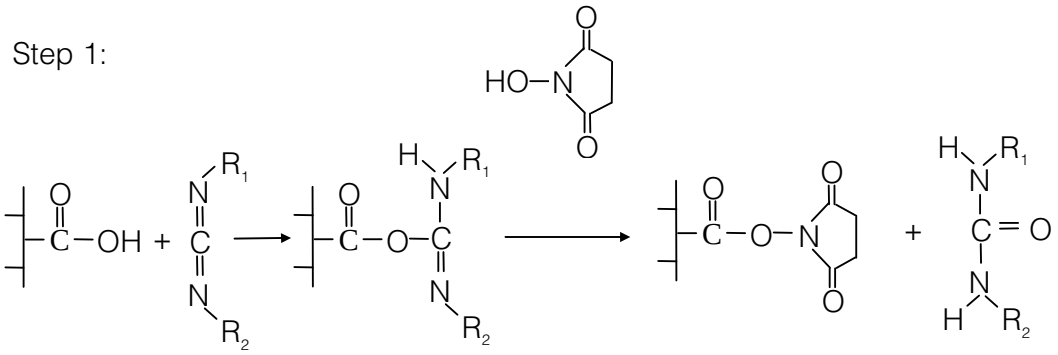
- Immobilization of capturing antibodies. Capturing antibodies are used when the activity of antibodies is reduced by the immobilization procedure. Detection of antigens is achieved in three steps. Firstly, immobilization of the capturing antibody (for example anti-Rabbit Anti Mouse-Fc). Secondly, binding of the second antibody (a mouse antibody) by the capturing antibody. Thirdly, specific binding of the antigen by the second antibody. Regeneration of the surface will usually break all non-covalent interactions.

These methods have been used to study biomolecular interactions intensively. Examples of biomolecular interactions are mentioned here:

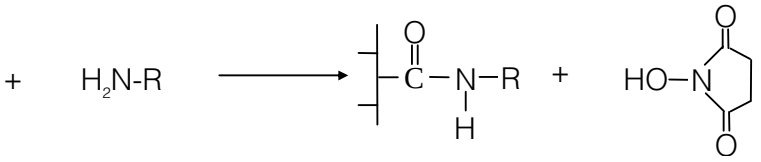
- Peptide-antibody interaction[18]
- Peptide-MHC interaction[19]
- Protein-antibody interaction, epitope mapping[20]
- Protein-DNA interaction[21]
- Protein-polysaccharide interaction[22]
- Protein-virus interaction[23]
- Protein-cell interaction[24]
- Protein-T cell receptor interaction[25]
- Antibody-antibody interaction, capturing antibody[26]
- DNA-DNA interaction[27]

The activated NHS-ester reacts with uncharged primary amino groups of biomolecules. This means that the reaction rate is favoured by high pH values of the buffer.
The reaction can only occur if the ligand is available for reaction, i.e. when it is inside the dextran layer. This is achieved by pre-concentration of the ligand. Positively charged biomolecules adsorb electrostatically to the negatively charged dextran layer by pre-concentration. Consequently, the ligand buffer should be lower than the isoelectric point (pI) of the ligand.
A compromise for pH values should be chosen for ligand solutions to fulfil the pre-concentration condition and the reaction rate condition for ligands.

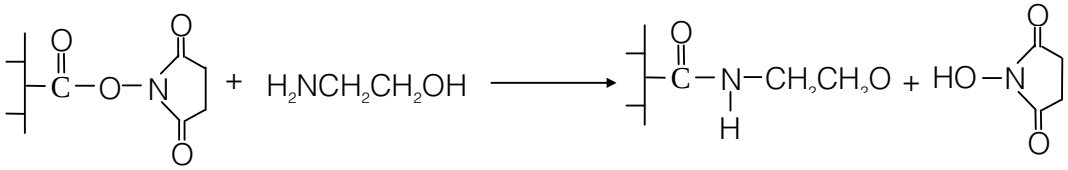
Step 1:



Step 2:



Step 3:



With R1 = $-CH_2CH_3$

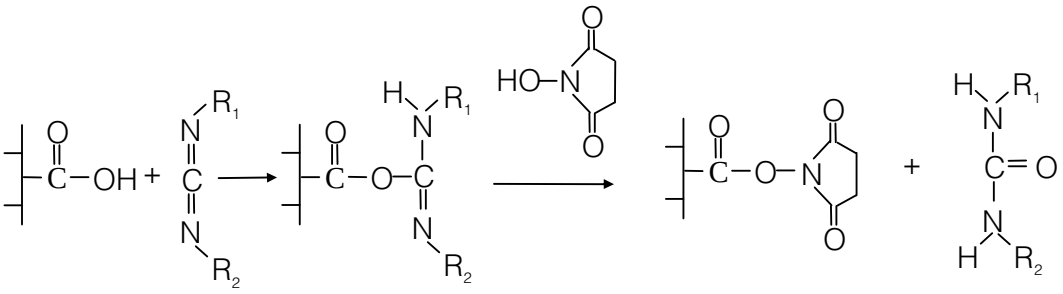
R2 = $-CH_2CH_2CH_2N^{\oplus}H(CH_3)_2\overset{\ominus}{Cl}$

R = biomolecule

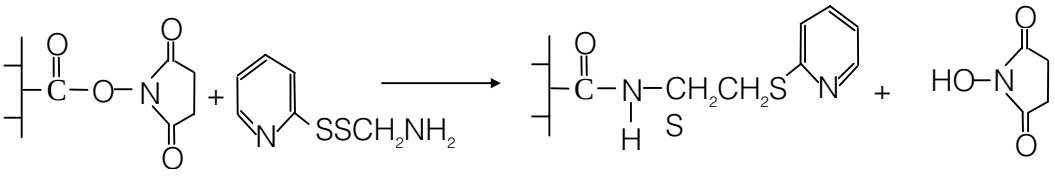
Immobilization by thiol functional groups of ligands is performed by a thiol coupling reagent:

1. activation of carboxymethyl groups to an NHS ester by EDC/NHS chemistry
2. reaction with 2-(2-pyridinyldithio)ethaneamine (PDEA) to introduce reactive disulfide bridges
3. reaction of disulfide bridges with thiol ligand groups
4. deactivation of excess disulfides with cysteine

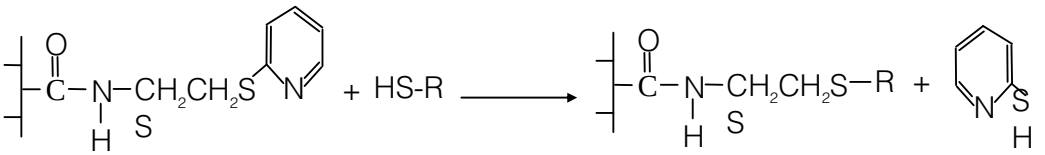
Step 1:



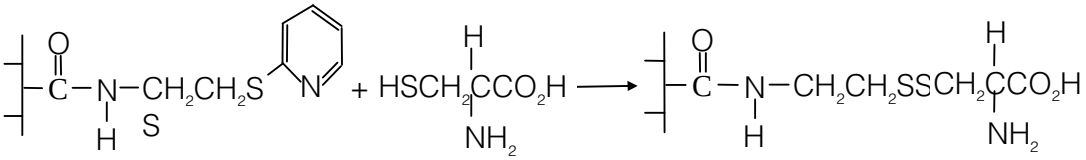
Step 2:



Step 3:



Step 4:



8.4.3 – Cuvette.

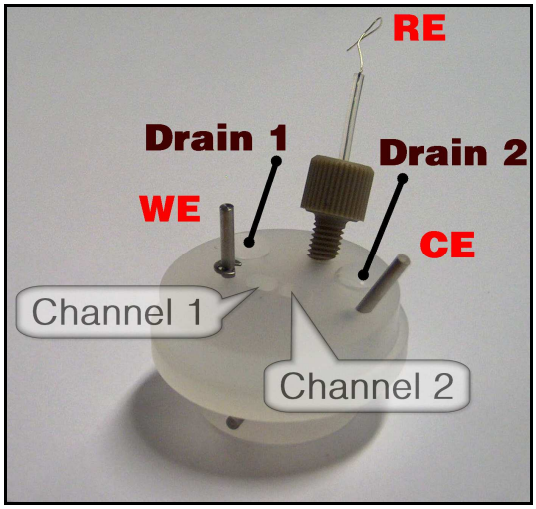


Figure 8.6 – The Electrochemical SPR Cuvette. With working (WE), reference (RE) and counter (CE) electrode connections.

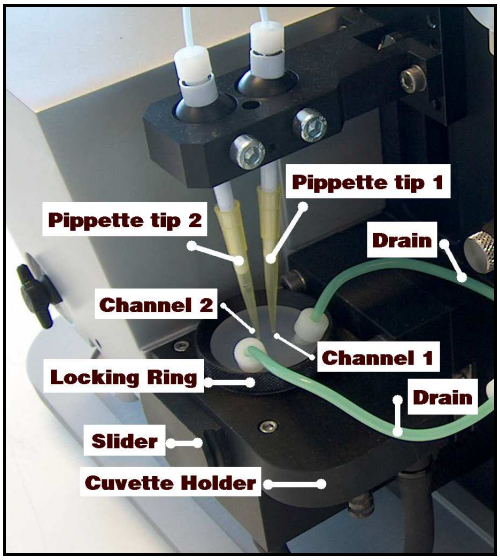


Figure 8.7 – The normal SPR Cuvette. Each pipette tip has an individual channel.

The cuvette limits the physical parameters for the reaction volume and position of the reaction on the gold layer. The disk (Eco Chemie B.V. standard supplied bare gold disk) and the sensor chip (Biacore supplied gold disk) should be installed onto different sliders (Figure 8.4). In essence, the cuvette is a multi-parameter controllable batch reactor, in which binding events take place at the bottom, at the sensor surface. To prevent concentration differences in the cuvette during measurements, the instrument is equipped with a controllable automatic aspirate-dispense mixing needle. A syringe is constantly aspirating and dispensing buffer into the cuvette during measurements to obtain reproducible hydrodynamic conditions. The hydrodynamic parameters of the cuvette are:

- mix volume
- speed or frequency of mixing
- distance of the pipette tip to the sensor surface
- volume of the solution in the cuvette
- diameter of the cuvette
- geometry of the needle
- viscosity of the solution
- temperature of the solution

Physical transport phenomena will determine how fast the biomolecular transport from a solution to the surface will be. Mass transport limitations

arise when the concentration of analyte at the sensor surface is lower than the total sample concentration. In the ESPR cuvette system, the mass transport to the surface is highly increased by the aspirate-dispense mixing process, which is a process according to the dynamic free wall-jet principle[10]. During a mixing cycle of set volume and set speed, the solution is aspirated from the cuvette. Normally, half the volume that is present in the cuvette will be aspirated followed by a dispense action. During the dispense action, a jet of the sample solution can be forced to flow into the diffusion layer of the surface. As a result, the mass transport can be increased enormously.
The cuvette is connected to a pump to drain the cuvette and to a pump to wash the cuvette.

8.4.4 – Liquid handling.

For fully automated measurements, the needles connected to the autosampler will aspirate and dispense all solutions. The autosampler is controlled by the Data Acquisition software. The liquid handling circuit is shown in Figure 8.8.

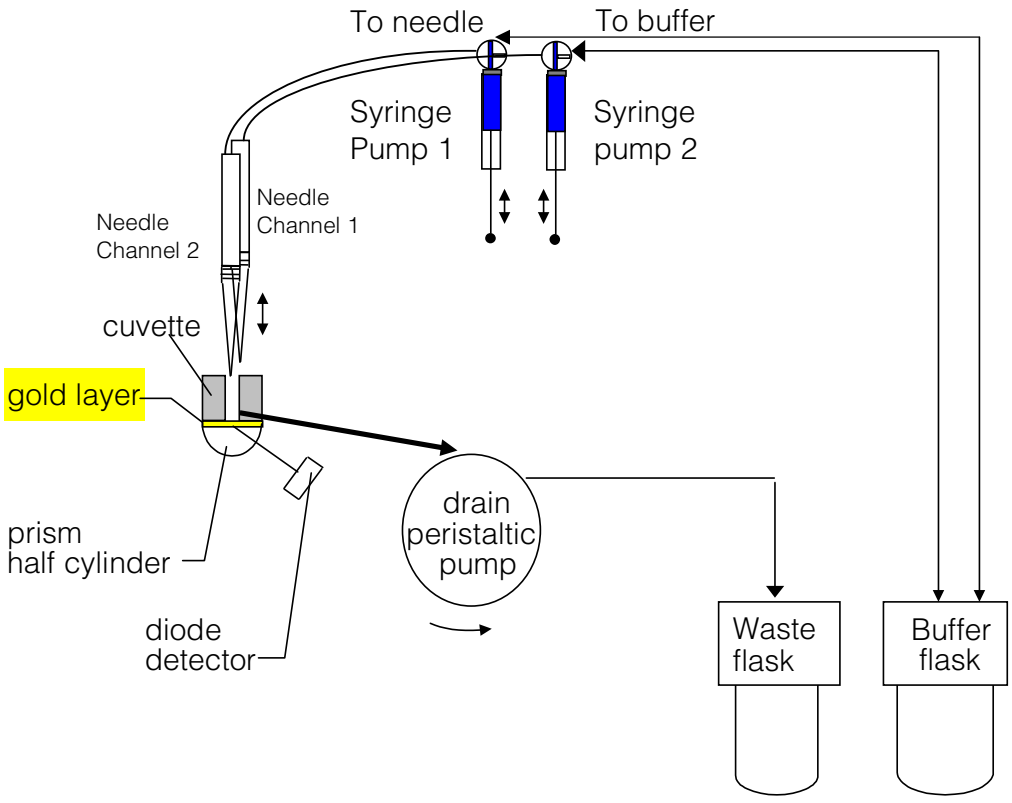


Figure 8.8 – Schematic picture of the instrument.
Two three-way valves, the pump 1 valve (channel 1) and the pump 2 valve (channel 2) determine the liquid handling. One peristaltic pump is used for draining and washing.

8.5 – SPR methods.

8.5.1 – Introduction.

Two approaches are available to study macromolecular interactions with the biosensor. The interactions can be studied directly or indirectly. Direct measurements monitor the binding of analytes with immobilized or coated ligands. A direct measurement can be performed in multiple steps. i.e. sequential binding of two or more components. If the molecular weight of an analyte is too low for detection, direct measurements are not possible.
In that case, binding of the analyte can be determined by an indirect measurement. Before a high molecular weight analyte is added to the immobilized or coated ligand, the binding sites are blocked by pre-incubation with a low molecular weight compound. The blocked analyte is not able to bind the ligand anymore, and therefore the binding curve will disappear partially or totally. With indirect measurements, the response is related to the amount of unblocked analyte and thereby to the amount of 'blocker' added during pre-incubation.
In an indirect measurement, two components compete with each other in a parallel process, and not in a serial process as in multiple direct measurements. The serial and parallel interaction processes can be combined freely. Direct and indirect measurements can be performed with the SPR disk and the sensor chip.

An important possibility of SPR is the determination of kinetic parameters of biomolecular interactions and determination of analyte concentrations. It is possible to separate kinetic measurements from concentration measurements. Consequently, kinetic measurements can be performed with non-purified analyte samples. These determinations will be explained in chapter 3 and4, together with an explanation of the kinetic evaluation software.
Methods for SPR measurements of the disk and the sensor chip are different, and are therefore summarized separately in the rest of this section.

8.5.2 – Methods using the SPR disk.

Measurements can be performed with sensor surfaces of bare gold or polymer coated sensor surfaces.

Bare gold surfaces:

- Macromolecular interaction measurements can be performed by coating the ligand electrostatically to the surface, followed by adding the analyte. After coating, a blocking compound is usually necessary to prevent a-specific interactions. This method is especially suitable for detection of large particles as cells and viruses[11].

- Biomolecular interaction measurements with biotinylated macromolecules[12,13]. First, the gold sensor surface is coated with biotin, followed by binding with streptavidin. Then, biotinylated molecules are allowed to bind with unoccupied binding sites of streptavidin (stoichiometry streptavidin-biotin interaction is 1:4). Finally, binding of the analyte can be measured.

- Biomolecular interaction measurements with thiol containing compounds. Gold interacts with sulfur[14]. By applying this property for peptides, self-assembled receptor layers were developed[15].

- Direct measurement of low molecular weight compounds by response enhancement with latex particles. Low molecular weight compounds can be attached to carboxy modified latex by a carbodiimide coupling reaction[16,17]. Direct binding of low molecular weight compounds coupled to latex particles can be determined using a coated ligand at the sensor surface.

Polymer coated surfaces:

- A thin film of 20-30 nm, of the polymer also used for ELISA microtiter plates, can be attached to the gold surface of the SPR disk by spincoating. ELISA methods can be used to study biomolecular interactions with the polymer-coated gold surface.

- Latex coating. Latex particles can be immobilized with ligand by a carbodiimide coupling reaction[16,17]. The latex particles can be coated on the SPR disk, resulting in a biospecific latex layer covering the sensor surface. An advantage of this layer is that total regeneration of the gold surface is possible with a sodium dodecyl sulphate (SDS) buffer solution.

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Chapter 9

9 – Maintenance.

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9.2 – Introduction.

In this chapter, the regular maintenance procedure is described.

During maintenance wear gloves, use clean lens paper and ultra pure cleaning solutions. If the instrument is contaminated with biohazards (like bacteria or viruses), disconnect all devices of the instrument that are exposed to the biohazard and clean them with the right cleaning agents for that biohazard. If any doubts exist about the cleaning procedure, please contact the local distributor.

9.3 – Storage of SPR disk and sensor chip.

There are three recommended procedures to store a disk or sensor chip:

- In the instrument during a relatively short period, e.g. until the next day. Store it in buffer with the lowest deactivating behaviour. If the immobilized biomolecules can resist distilled water, this is preferred. Otherwise, an ammonium carbonate buffer may be used, because the salts of the buffer will evaporate. In order to reduce the evaporation of the solution from the cuvette, put some Parafilm on the cuvette.
- In the slider during a relatively short period of maximal a week. Wash the cuvette with distilled water. (Never wash with buffer because salts will dry and will destroy the coating). Drain the cuvette and disconnect the cuvette. Remove the slider from the instrument. Place the slider in a plastic bag and store the slider in the bag in the refrigerator. The plastic bag is necessary because otherwise moisture will condense on the hemi-cylinder lens. Let the slider equilibrate at room temperature before removing the plastic bag (ca 30 minutes). Place the slider with disk or chip in the holder and place the cuvette.
- For storage of biomolecules on the disk or chip for a longer period, first wash the coating with distilled water to remove the buffer containing salts. Remove the cuvette and slider from the instrument and remove the disk or chip from the slider. Place the disk with a pair of tweezers in a storage box. The sensor chip can be inserted in the sensor chip cover. Place the sensor chip or disk in a storage box or tube and add some silica gel bags. Close the box or tube and store it in the refrigerator for a longer period.

Always check the bioactivity after storing.

9.4 – Optics.

- Make it a routine to check the shape of the SPR-dip with the update SPR command, in order to verify the right quality of the sensor disk, the matching of the disk with the hemi-cylinder and the cleanness of the optics. The possible errors in the SPR-dip check that may occur are described in chapter 10.

9.5 – Routine inspections.

Inspect all visible liquid connections; pump syringes and valves, needles, drain and wash pump connections. If any leaks are discovered, clean and tighten the connections or replace tubing and seals if necessary.

Check the piston of the syringe pumps at least once a month. Look for bacterial growth or salt crystals. Check the tubing of the peristaltic pumps on signs of wear.

9.6 – Replacing syringe and piston.

The seal of each syringe should be changed with a minimum of once per year. Seal lifetime varies according to the application, fluids used and quality of maintenance. Cleaning the syringe at least once every three months should extend the lifetime of the seal.
Screw the syringe from the valve port. Screw the piston from the manifold. Fill a new syringe with distilled water before replacing. Carefully eliminate air bubbles in the syringe and replace the syringe.

Chapter 10

10 – Troubleshooting.

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The following tables address problems that may be encountered with common methods of immuno-detection and real-time interaction sensing with the Autolab Twingle. Typical errors or misinterpretations are also presented. For serious problems not found in this chapter, please contact the local distributor.

10.2 – Troubleshoot list – general.

Problem	Possible causes	Suggested solution
The entire instrument is not working.	Fuse defect. No mains power.	Replace fuse if source is known. Check for proper mains voltage.
The software is not working.	Wrong installation or combination of the SPR files. Win98/2000/XP not properly installed.	Install with the most up-to-date version of the software. First rename the SPR directory and delete the SPR icons. See Chapter 2 for installation instructions.
The software is not working in combination with the Twingle.	The instrument is not connected to the host computer. Serial ports are not correct. The serial cable is defect.	First, start the SPR program on the host computer, then switch on the instrument. Use preferably COM2 for the serial cable to connect the Twingle.
SPR starts, but there is no initialisation sound of autosampler and syringe pumps.	Internal fuse defect.	Call the local distributor.
Status bar indicates “not connected”.	The link between the internal PC and the host computer is not working. RS232 cable not connected properly or is defect.	Connect serial cable between Com port of computer and Com port of Twingle. Check communications menu for port settings.
The minimum of the SPR dip is bad. Worse than 10% of the maximum intensity.	Coatings are too thick or not homogeneous. The thin gold layer is not clean or damaged. Wafer or sensor chip is out of specs.	Try a clean wafer or sensor chip. Change the coating procedures to higher spinning rates and lower concentrations of polymer in solvent.
The minimum of the	Particles will adhere	Filter the sample solution. Try

Problem	Possible causes	Suggested solution
SPR dip changes dramatically during a measurement.	to the surface. Inhomogenities in the coating are created during an experiment.	another coating procedure.
Signal is too noisy.	Bad SPR dip. Interval time is too small. Optics are not clean.	Clean the surface with SDS in aqua distilled water followed by 96% alcohol and wash with buffer containing SDS. Check SPR dip. Use an alternate SPR wafer. Increase the interval time. Clean the hemi-cylinder.
The SPR check has some intensity variations.	The hemi-cylinder is not clean.	By turning the spindle the intensity variations remain at the same angle, clean either the hemi-cylinder or optics. For a thorough cleaning of the optics and lining out of optics please contact the local distributor.
Strange behaviour around an angle of zero mdegree when water is replaced by PBS.	Only a stepwise change of the bulk refractive index should be measured. The SPR dip is almost out of the dynamic range	First adjust the spindle to 1500 mdegree (set interval time on 0.5 seconds) and then adjust spindle slowly to around zero mdegree.
Drift of the baseline signal.	Temperature in the laboratory is not constant. There is a reciprocal correlation of temperature and angle shift. The coating is not stable.	Place SPR in a climate chamber. A spincoating of e.g. polystyrene should first be adapted to the new buffer.

10.3 – Troubleshoot list - sample handling.

Problem	Possible causes	Suggested solutions
The volume in the cuvette decreases during an experiment.	There is a leakage in one of the chambers of the cuvette. The cuvette is damaged. The cuvette is not properly mounted and is askew. There is evaporation of sample during long experiments.	Try another cuvette. Turn the mounting screw stepwise a little after each other, in order to press down the cuvette as a whole, perpendicular to the wafer. Prevent evaporation with a cover.
The signal is not stable and noisy.	Air bubbles or solids are interfering with the mixture of the sample solution.	Mix with a decreased volume.
No mixing occurs or mixing falls out. There is an irreproducible SPR angle shift.	The needle tubing is not mounted properly on the needles. The tubing has a leakage.	Improve the tube connections on the syringe pump and on the needles.
Back plate and ground floor in are wet.	Leakage of pump valve or piston seal syringe. Connection of tubing is not fitted properly. Tubing is damage.	Change pump valve, piston seal or syringe mounting fittings. Check and clean tube connections. Change defective tubing.
Unstable baseline.	Temperature is not stable, air in liquids, unstable flow or mixing, leakage at tube connections, piston wear out.	View temperature and check stability. De-gas if air in the liquid is the cause. Check the connections on leakage. Check the quality of the piston of the pump.
No response after injection.	Clogging of the needle or too loosely fitted tubing on the needle.	Check connections and change the direction of flow. Clean the needle.
The signal has a regular noise with time scale of the interval time.	The pump frequency is in phase with the interval time.	Increase the frequency of the pump speed vs. pump volume e.g. twice the interval time.

Problem	Possible causes	Suggested solutions
There is a big spike after injection of the sample.	The bulk refractive indices of the injection and the starting buffer solution are not the same.	Try to dilute the sample solution with the starting buffer. Look for differences in composition of the solutions. Try another injection sequence.
A big shift of the baseline occurs after washing. Especially occurring when a disk with polystyrene coating is applied.		Wash with the help of the sequencer. Add fresh buffer immediately after draining of the sample from the cuvette.

10.4 – Troubleshoot list - biochemistry, hydrodynamics, coatings.

Problem	Possible causes	Suggested solutions
No signal or weak signal.	Reagents were omitted or added in an incorrect order. Incorrect reagents were used.	Use all reagents in the proper sequence. Use matched reagents (for example, a mouse primary antibody with an anti-mouse secondary antibody).
No signal or weak signal.	Insufficient amounts of antigen were present.	Increase the primary antibody concentration. Increase the interaction time of the primary antibody with the antigen. Use more antigen.
	Improper storage of reagents resulted in degradation.	Store reagents at recom-mended conditions.
	Low affinity primary anti-body was lost	Try higher affinity antibody if available.

Problem	Possible causes	Suggested solutions
	during immuno-detection procedure.	Increase incubation time or concentration of primary antibody with antigen to maximize the amount of primary antibody bound. Decrease wash volume and time to minimize dissociation of primary antibody.
	Primary antibody reacted poorly with denatured antigen. Incubation times with secondary antibody or the streptavidin or avidin conjugate were insufficient.	Use procedures for retention of the native form of the antigen. Increase the incubation times.
High non-specific adsorption.	Blocking was insufficient.	Increase concentration of blocking agent. Increase incubation time with blocking agent. Try to alternate blocking agent
	Reagents were too concentrated.	Dilute primary antibody, secondary antibody, and/or streptavidin or avidin conjugate.
Excessive signal.	Concentration or amount of reagents used was excessive. Excessive incubation times were used.	Dilute reagents to reduce signal. Decrease the amount of antigen employed. Decrease incubation times.
Poor reproducibility of the results.	Contamination of the cuvette or interfering substances resulted in variable signals. During mixing, air bubbles are aspirated in the needle.	Increase wash volumes to remove residual reagents more effectively. Use the right cleaning conditions for the cuvette. Use lower mixing volumes. Prevent evaporation of the sample.

Problem	Possible causes	Suggested solutions
High injection spike.	The bulk refractive index of the injected sample is too high. The temperature of injected sample is different from ambient.	Dilute the injected sample. Add (blocking or glucose) components to the initial buffer to reduce injection spike. Wait 5 minutes before injection of the sample solution and let temperature come to ambient.
Reaction too slow or too fast.	Concentration, mix frequency, volume, needle distance. Too viscous sample.	Increase concentration. Increase mixing frequency.
Bad SPR dip.	The primary coating is too thick or is too irregular or rough.	Change the spin coat conditions. RPM and/or concentration of the polymer solvent.
Shape of SPR-dip changes during measurement.	Particles in the sample solution. Agglutination at the surface as a result of denaturation conditions or interacting debris or other components. Change of roughness of the surface.	Filter the sample solution prior to addition. Use other buffers or coating procedures. Try another blocking agent. Try another coating polymer. Increase wash volumes to remove residual reagents more effectively.

10.5 – SPR signal problems.

Signal problems in SPR occur when the minimum of an SPR dip is not determined correctly. Before starting a measurement, an SPR dip check should be performed to confirm the right conditions.
Below some SPR dip checks are shown for certain problems that could occur during SPR measurements. In case of an abnormal SPR dip, please follow the instructions below. In practice, a SPR dip varies slightly. This means, that the SPR dips shown in this section may differ slightly from dips produced with your instrument.

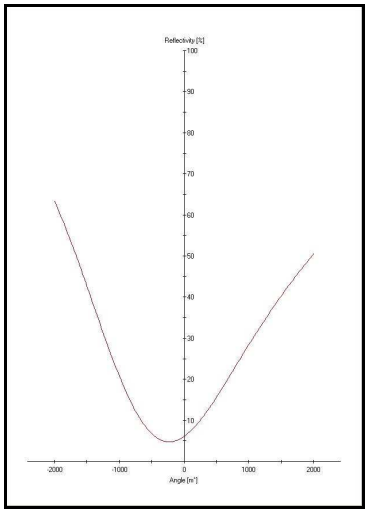

Figure 10.1 – Ideal dip.

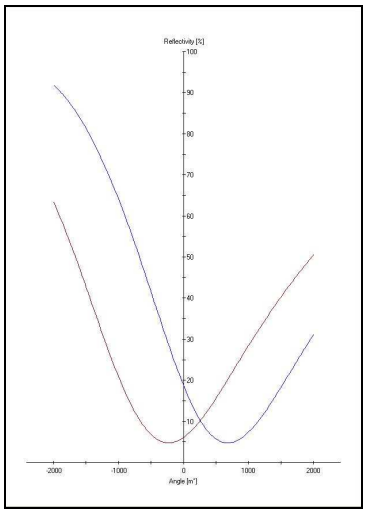

Figure 10.2 – SPR dip shift.

Ideal dips are smooth and symmetrical at the bottom of the dip. At the beginning of a measurement, the SPR dip is located at the x-axis at zero degree (Figure 9.1) and at the y-axis zero degree. The absolute intensity of an SPR dip is normally lower than 10 %. If it is above 10 %, air bubbles are present in most cases. Upon binding, the SPR dip will shift to the right as shown in Figure 9.2.

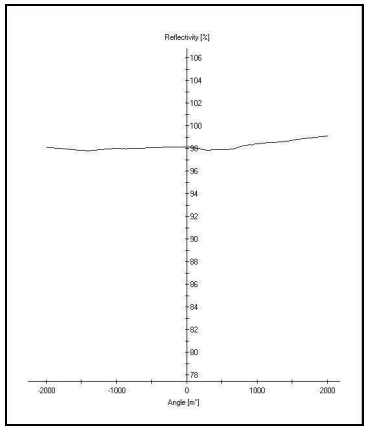

Figure 10.3 – No SPR dip.

Problem	Possible cause	Suggested solutions
No SPR dip (Figure 10.3).	Incorrect spindle position.	Adjust spindle position.
	Incorrect scale settings.	Adjust scale.
	Incorrect slider installation: 1. hemi-cylinder not clean 2. no immersion oil 3. disk reversed inserted (gold layer not in buffer compartment) 4. damaged gold layer	clean cylinder repeat installation repeat installation new disk/chip
	Incorrect cuvette installation: 1. cuvette not centered on laser spot 2. no buffer in cuvette 3. air bubbles between buffer and gold layer	repeat cuvette installation add buffer in cuvette drain cuvette and inject buffer
	Optics: 1. no laser light 2. laser spot is not centered on the hemi-cylinder 3. other	Contact the local distributor

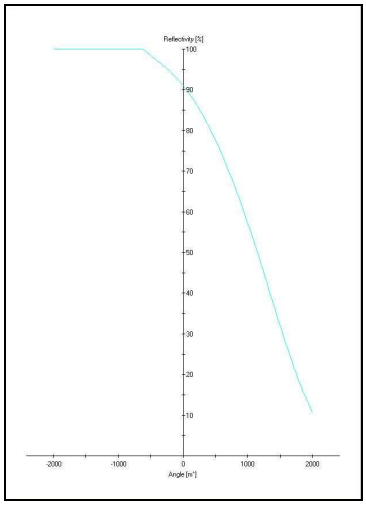

Figure 10.4 – SPR dip shifted right.

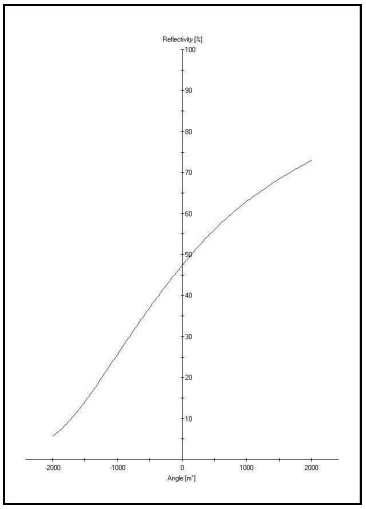

Figure 10.5 – SPR dip shifted left.

Problem	Possible cause	Suggested solutions
SPR dip shifted right (Figure 10.4)	Incorrect spindle position and SPR signal out of range	Adjust spindle to SPR signal of approximately zero degrees (Figure 9.1)
SPR dip shifted left (Figure 10.5)	Incorrect spindle position and SPR signal out of range	Adjust spindle

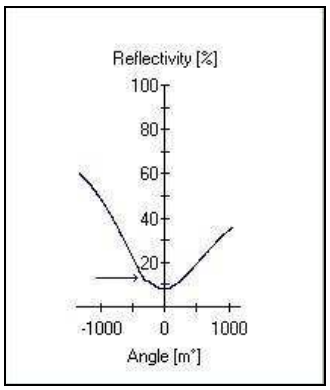

Figure 10.6 – Unsymmetrical SPR dip.

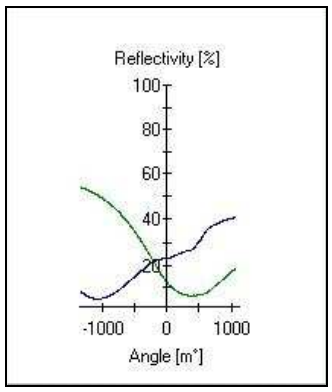

Figure 10.7 – Consequences of an unsymmetrical SPR dip.

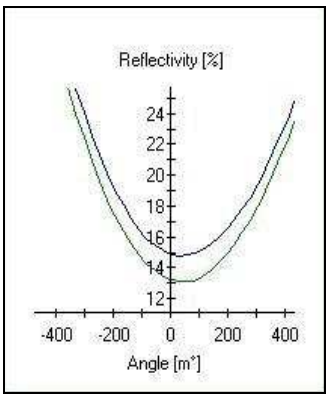

Figure 10.8 – SPR dip shifted up.

Problem	Possible cause	Suggested solutions
Unsymmetrical SPR dip (fig.10.6, indicated by arrow)	1. Particles in buffer/sample 2. Dirt on hemi-cylinder 3. Dirt in immersion oil 4. Dust in optics	Filter buffer/sample Clean half cylinder New immersion oil Clean optics, please call the local distributor for advise
Unsymmetrical SPR dip, consequences (fig 10.7): Noisy signals, when a new SPR dip is located at the disturbance of the first dip	see above	see above
SPR dip shifted up (intensity minimum > 10 %) SPR curve also broader	1. Particles on surface 2. Air bubbles in cuvette (hydrophobic surfaces)	Wash cuvette thoroughly Wash cuvette thoroughly, avoid to drain the cuvette completely for hydrophobic surfaces like bare gold and polystyrene coatings

Chapter 11

11 – Figures.

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